

Physico-chemical Study of Dye Effluent and Its Impact on Soil and Nodulation in Some Leguminous Plants

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By

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2020

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ABSTRACT

Many small dyeing and printing industries in Kaithun discharge their effluents directly or indirectly into land and water streams. In the present study, effluent from these industries was analyzed for various Physico-chemical parameters like temperature, pH, conductivity, hardness, total dissolved solids, Biological Oxygen Demand, Chemical Oxygen Demand and heavy metals. The contaminated wastewater has been used to irrigate the agricultural fields. Similarly, physico-chemical characters of effluent contaminated soil were analyzed and the soil parameters were found comparatively higher in the field irrigated with effluent contaminated water. In the present work the effect of dyeing and printing effluent on seed germination, seedling growth, fresh weight and dry weight of seedlings, biochemical, yield and nodulation parameters of two different leguminous plants (*Glycine max L.* and *Medicago sativa L.*) were also evaluated. It was found that effluent had both stimulatory as well as inhibitory effects. The degree of enhancement and inhibition depended upon the concentrations of effluent. It was found that root growth was highly affected than shoot growth. Thus roots of the tested crops were highly sensitive to effluent treatment than shoots. Among all the studied biochemical constituents pigment content was found to decrease (due to inhibition of chlorophyll biosynthesis) with increasing effluent concentrations in both the studied leguminous plants. The free sugar in both plants was found to decrease in higher concentration of dyeing and printing effluent. Leguminous plants show increasing trend of protein content in the treated pots (T_1) in comparison to the control and decrease in higher concentration of dyeing and printing effluent. The reasons behind such reduction of protein and amino acid content at higher concentrations of effluent are due to the decrease the synthesis of protein and increase the rate of protein degradation. The relative degree of toxicity showed that *Medicago sativa L.* was highly susceptible to effluent treatment and *Glycine max L.* was relatively more resistant. Moreover, it was also found that the plants productivity and nodulation on pots irrigated with effluent contaminated water was relatively low at higher concentrations (T_3 , T_4 and T_5 treatment level) of effluent than on pots irrigated with uncontaminated (Tap) water and at treatment level

T_1 and T_2 . In this present research work bacterial strains were also isolated from dyeing and printing effluent contaminated soil samples and three Bacterial isolates such as *Bacillus subtilis*, *Bacillus cereus* and *Pseudomonas aeruginosa* were isolated and identified in contaminated soil of Kaithun region and effluent treated soil sample from Pot experiment. Two *Rhizobium* i.e. *Rhizobium japonicum* (*Glycine max L.*) and *Rhizobium meliloti* (*Medicago sativa L.*) were also isolated from root nodules of both the experimental plants treated with 20% concentrated effluent (T_1) and control.

CANDIDATE'S DECLARATION

I, hereby, certify that the work, which is being presented in the thesis, entitled "***Physico-chemical study of Dye effluent and Its Impact on Soil and Nodulation in some Leguminous plants***" partial fulfillment of the requirement for the award of the Degree of Doctor of Philosophy, carried under the supervision of Dr. Azra Akhtar and submitted to the University of Kota, Kota represents my ideas in my own words and where others ideas or words have been included. I have adequately cited and referenced the original sources. The work presented in this thesis has not been submitted elsewhere for the award of any other degree or diploma from any Institutions.

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Pratibha Mahawar

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(Pratibha Mahawar)

Research Scholar

CONTENTS

<i>S. No.</i>	<i>Chapter Name</i>	<i>Page No.</i>
1.	Introduction	1-21
2.	Review of Literature	22-44
3.	Materials and Methods	45-86
4.	Observation and Results	87-155
5.	Discussion	156-177
6.	Conclusion	178-182
7.	Summary	183-192
8.	Bibliography	193-219
	Publications	220-238

LIST OF TABLES

S. NO.	PARTICULARS	Page No.
1.	Tolerance limits for discharge of industrial effluents prescribed by BIS.	9
2.	Various classes of Dyes	10
3.	Ladpura Kaithun- Village Overview	19
4.	List of Sampling Sites	21
5.	Various Types of Media Composition	86
6.	Physico-chemical characteristics of dyeing and printing effluent of the study area	91
7.	Effect of the dyeing and printing effluent on soil characteristics	93
8.	Effect of dyeing and printing effluent on germination and seedling growth of <i>Glycine max</i> L. : (Petri plate Method)	96
9.	Effect of dyeing and printing effluent on seedling growth of <i>Glycine max</i> L. after 30 day of sowing	107
10.	Effect of dyeing and printing effluent on seedling growth of <i>Glycine max</i> L. after completion of life cycle (60 DAS)	108
11.	Effect of dyeing and printing effluent on pigment contents (mg g ⁻¹ fresh weight) of <i>Glycine max</i> L. after 30 day of sowing	109
12.	Effect of dyeing and printing effluent on pigment contents (mg g ⁻¹ fresh weight) of <i>Glycine max</i> L. after completion of life cycle (60 DAS)	110
13.	Effect of dyeing and printing effluent on Yield parameters of <i>Glycine max</i> L. after completion of life cycle (60 DAS)	111
14.	Effect of dyeing and printing effluent on biochemical contents (mg g ⁻¹ fresh weight) of <i>Glycine max</i> L. after completion of life cycle (60 DAS)	112
15.	Effect of dyeing and printing effluent on Nodulation in <i>Glycine max</i> L. after completion of life cycle (60 DAS)	115
16.	Effect of dyeing and printing effluent on Leghaemoglobin content (mg g ⁻¹ fresh nodule) of <i>Glycine max</i> L. treated with dyeing and printing effluent	116
17.	Effect of dyeing and printing effluent on germination and seedling growth of <i>Medicago sativa</i> L.: (Petri plate Method)	119

18.	Effect of dyeing and printing effluent on seedling growth of <i>Medicago sativa</i> L. after 30 day of sowing	130
19.	Effect of dyeing and printing effluent on seedling growth of <i>Medicago sativa</i> L. after completion of life cycle (60 DAS)	131
20.	Effect of dyeing and printing effluent on pigment contents (mg g ⁻¹ fresh weight) of <i>Medicago sativa</i> L. after 30 day of sowing	132
21.	Effect of dyeing and printing effluent on pigment contents (mg g ⁻¹ fresh weight) of <i>Medicago sativa</i> L. after completion of life cycle (60 DAS)	133
22.	Effect of dyeing and printing effluent on biochemical contents (mg g ⁻¹ fresh weight) of <i>Medicago sativa</i> L. after completion of life cycle (60 DAS)	134
23.	Effect of dyeing and printing effluent on Yield parameters of <i>Medicago sativa</i> L. after completion of life cycle (60 DAS)	135
24.	Effect of dyeing and printing effluent on Nodulation of <i>Medicago sativa</i> L. after completion of life cycle (60 DAS)	137
25.	Effect of dyeing and printing effluent on Leghaemoglobin (mg g ⁻¹ fresh nodule) of <i>Medicago sativa</i> L. after completion of life cycle (60 DAS)	138
26.	Microbiological Analysis of Soil	140
27.	Morphological Result	141
28.	Biochemical Test and Identification of <i>Pseudomonas aeruginosa</i>	143
29.	Biochemical Test and Identification of <i>Bacillus subtilis</i>	145
30.	Biochemical Test and Identification of <i>Bacillus cereus</i>	147
31.	Morphological, Cultural and Biochemical Characteristics of Isolated Soil Bacteria	148
32.	Cultural Morphological and Biochemical Character of <i>Rhizobium japonicum</i> (<i>Glycine max</i> L.)	151
33.	Cultural Morphological and Biochemical Character of <i>Rhizobium meliloti</i> (<i>Medicgo sativa</i> L.)	152
34.	Cultural, Morphological and Biochemical Characterstics of Isolated <i>Rhizobium</i>	155

LIST OF FIGURE

S. NO.	PARTICULARS
1.	Graph showing comparative study of physico-chemical characteristics of effluent and control
2.	Graph showing comparative study of physico-chemical characteristics of effluent and control
3.	Graph showing comparative study of physico-chemical characteristics of effluent and control
4.	Graph showing comparative study of physico-chemical characteristics of contaminated soil
5.	Graph showing comparative study of physico-chemical characteristics of contaminated soil
6.	Graph showing comparative study of physico-chemical characteristics of contaminated soil
7.	Graph showing effect of different treatment levels of dyeing and printing effluent on various parameters in <i>Glycine max L.</i> (Petri-Plate Method)
8.	Graph showing effect of different treatment levels of dyeing and printing effluent on various growth parameters in <i>Glycine max L.</i> (Petri-Plate Method)
9.	Graph showing effect of different treatment levels of dyeing and printing effluent on various growth parameters in <i>Glycine max L.</i> (Pot Experiment)
10.	Graph showing effect of different treatment levels of dyeing and printing effluent on various growth parameters in <i>Glycine max L.</i> (Pot Experiment)
11.	Graph showing effect of different treatment levels of dyeing and printing effluent on various growth parameters in <i>Glycine max L.</i> (Pot Experiment)
12.	Graph showing effect of different treatment levels of dyeing and printing effluent on Pigment content in <i>Glycine max L.</i> (Pot Experiment)
13.	Graph showing effect of different treatment levels of dyeing and printing effluent on various growth parameters in <i>Glycine max L.</i> (Pot Experiment)
14.	Graph showing effect of different treatment levels of dyeing and printing effluent on nodulation parameters in <i>Glycine max L.</i> (Pot Experiment)

15.	Graph showing effect of different treatment levels of dyeing and printing effluent on various growth parameters in <i>Medicago sativa</i> L. (Petri-Plate Method)
16.	Graph showing effect of different treatment levels of dyeing and printing effluent on various growth parameters in <i>Medicago sativa</i> L. (Petri-Plate Method)
17.	Graph showing effect of different treatment levels of dyeing and printing effluent on various growth parameters in <i>Medicago sativa</i> L. (Pot Experiment)
18.	Graph showing effect of different treatment levels of dyeing and printing effluent on various growth parameters in <i>Medicago sativa</i> L. (Pot Experiment)
19.	Graph showing effect of different treatment levels of dyeing and printing effluent on various growth parameters in <i>Medicago sativa</i> L. (Pot Experiment)
20.	Graph showing effect of different treatment levels of dyeing and printing effluent on Pigment content in <i>Medicago sativa</i> L. (Pot Experiment)
21.	Graph showing effect of different treatment levels of dyeing and printing effluent on various growth parameters in <i>Medicago sativa</i> L. (Pot Experiment)
22.	Graph showing effect of different treatment levels of dyeing and printing effluent on nodulation parameters in <i>Medicago sativa</i> L. (Pot Experiment)

LIST OF PLATES

S. NO.	PARTICULARS
1.	(A) Study Site in Rajasthan Map (B) Study Site in Google Map
2.	Weaving Process
3.	Printing Process
4.	Various Effluent discharge sites
5.	Preparation for Printing Process
6.	Experimental Plants
7.	Various Parts of both the Experimental Plants
8.	Seeds of Selected legume Plants for Study
9.	Showing Percentage Germination of <i>Glycine max</i> L. in Different Treatment Levels of Dyeing and Printing Effluent (Petri-Plate Method)
10.	Pot Experiment (<i>Glycine max</i> L.)
11.	Showing Percentage Germination of <i>Medicago sativa</i> L. in Different Treatment Levels of Dyeing and Printing Effluent (Petri-Plate Method)
12.	Pot Experiment (<i>Medicago sativa</i> L.)
13.	Root System in Both the Experimental Plant
14.	Bacterial Population in Contaminated Soil Samples
15.	Biochemical Reaction by <i>Pseudomonas aeruginosa</i>
16.	Biochemical Reaction by <i>Bacillus subtilis</i>
17.	Biochemical Reaction by <i>Bacillus cereus</i>
18.	Various Stages of Rhizobium Culture in <i>Glycine max</i> L.
19.	Various Stages of Rhizobium Culture in <i>Medicago sativa</i> L.
20.	Tubes Showing Biochemical Reaction by Rhizobial Strains of <i>Glycine max</i> L.
21.	Tubes Showing Biochemical Reaction by Rhizobial Strains of <i>Glycine max</i> L.
22	Tubes Showing Biochemical Reaction by <i>Bacillus subtilis</i> from Control treatment level of <i>Glycine max</i> L.
23	Tubes Showing Biochemical Reaction by <i>Pseudomonas aeruginosa</i> from 20% (T ₁) treatment level of <i>Glycine max</i> L.

24	Tubes Showing Biochemical Reaction by <i>Bacillus subtilis</i> from 20% (T ₁) treatment level of <i>Glycine max</i> L.
25	Tubes Showing Biochemical Reaction by Rhizobial Strains of <i>Medicago sativa</i> L.
26	Tubes Showing Biochemical Reaction by Rhizobial Strains of <i>Medicago sativa</i> L.
27	Tubes Showing Biochemical Reaction by <i>Bacillus subtilis</i> from Control treatment level of <i>Medicago sativa</i> L.
28	Tubes Showing Biochemical Reaction by <i>Bacillus subtilis</i> from Control treatment level of <i>Medicago sativa</i> L.
29	Tubes Showing Biochemical Reaction by <i>Bacillus cereus</i> from 20% (T ₁) treatment level of <i>Medicago sativa</i> L.
30	Tubes Showing Biochemical Reaction by <i>Bacillus subtilis</i> from 20% (T ₁) treatment level of <i>Medicago sativa</i> L.

LIST OF ABBREVIATIONS

- BP - Between Paper
- BOD - Biological Oxygen Demand
- COD - Chemical Oxygen Demand
- EC - Electrical Conductivity
- EDTA - Ethylene Diamine Tetraacetic Acid
- HCl - Hydrochloric Acid
- HNO₃ - Nitric Acid
- IUCN - International Union for Nature Conservation
- K₂Cr₂O₇ - Potassium Dichromate
- MnSO₄ - Manganous Sulphate
- MR - Methyl Red
- NaOH - Sodium Hydroxide
- NAM - Nutrient Agar Media
- OM - Organic Matter
- TDS - Total Dissolved Solids
- VP - Voges Proskauer
- WHC - Water Holding Capacity
- WHO - World Health Organization
- YEMA - Yeast Extract Mannitol Agar

CHAPTER – 1



INTRODUCTION

INTRODUCTION

Industrialization plays a major role for the economic development of any nation. The industries play an important role for the development of Indian economy and employment generation. However, the industrial development sometime creates adverse effect on the human population, water, air, soil and environment. These industries produce lots of chemical waste in solid and liquid forms, which are disposed of in the adjacent land. These chemical wastes contain heavy metals and many harmful substances, which affect the physical, chemical and biological properties of soils and also contaminate surface water. Industrial discharge has a various types of pollutants with chemical constituents of undesirable concentration which can deteriorate the surface and ground water resources. The wastewater/effluent treatment system in Indian industries are endorse to be essentially installed to meet the waste water discharge norms, but presently only 10% of the wastewater/effluent generated is treated and the rest of untreated/polluted water is discharged into nearby water bodies.

Most of the industries are using water as a resource and in turn discharge high amount of wastewater, which have high BOD and COD values and contain hazardous substances like heavy metals. Wastewater treatment process is either non-existent or is inefficient, so the untreated or partially treated wastewater is allowed to release directly into the water streams. Due to the lack or scarcity of water this untreated and partially treated polluted water used for the irrigation purpose. Long term irrigation with polluted wastewater/effluent causes the contamination of soil. These pollutants flow through the food chain thus ultimately causes harmful effects on human and animal health.

Effluents from various types of industries are normally considered as the most hazardous industrial pollutants containing organic and inorganic compounds, acids, alkalies, suspended solids and other materials. Disposal of wastewater or effluent has become a global concern as the industries are associated with the generation of high volumes of effluents, when untreated effluents are discharged

Introduction

into the environment; it disrupts the ecosystem of living organisms. The disposal of waste water is a major concern confronted by districts, especially within the case of metropolitan areas with limited space for land based treatment and disposal.

Environmental degradation has now become a global challenge and maintaining ecosystem health is a serious issue being confronted by the environmentalists. Almost all industries are seen to discharge their wastes into water and on land without any treatment or after partial treatment. Even at the places where some treatment facilities exist, these are not being operated properly. Resultantly these waste waters or effluents pollute the water resources and ultimately the agricultural land.

Textile printing is one of the oldest handicrafts. The intrinsic beauty of textile is enhanced by surface ornamentation with multi colored effect. The ornamentation in fabric can be achieved by various methods, of which dyeing and printing are the most popular and extensively used. Textile printing differs essentially from dyeing in that it is designed to produce multi colored patterns on textile material rather than a solid pattern over a single cover in a specified area. Textile industries contribute significantly toward the economy of developing countries like India. The textile industry provides jobs without higher skills, which in turn place a main role in providing employment in poor and developing countries. The textile industries in India deliver employment opening to 35 million people of the country.

Textile is an important industry for Rajasthan, representing over 20 percent of the investment made in the state. Rajasthan contributes over 7.5 percent of India's production of cotton and blended yarn and over 5 percent of fabrics. In Kaithun region, Kota (Raj.) approximately 250 block and screen printing units are situated. A number of Azo dyes are used in dyeing and printing units at kaithun and in turn a high volume of untreated/polluted waste water released at various steps of dyeing and printing and discharged into drains and open lands adjoining the printing units.

1.1 Major Problem: Textile dyeing and printing effluent as a pollutant

Due to increasing industrialization, lots of chemicals including dyes are manufacturing and use in day to day life. Dyes usually have a synthetic origin and complex aromatic molecular structure which make them more difficult to biodegrade. Approximately 10,000 different dyes and pigments are used industrially and over 0.7 million tons of synthetic dyes are produced worldwide. Due to modern life style increasing urbanization and development the use of dyes and dye based products growing now these days. Once they enter an ecosystem, dye based products initiates a series of processes, affecting both it biotic and abiotic elements. Heavy metals in the colorants used in printing industry have been reduced significantly in the past 20 years but many are still in use. For example titanium oxide, chromate, molybdenum and iron are used as pigment, titanium oxide is used for pearlescent pigment and aluminum and bras are used in metallic inks. Heavy metals pose unique and very serious environmental problem. Unfortunately, most of the dyes escape conventional waste water treatment and persist in the environment as a result of their high stability to light, temperature and water. Dyes can remain in the environment for a long period of time because of high thermal and photo stability to resist biodegradation.

Dye molecules consist of two main components namely the chromophores, liable for generating the color and the auxochromes, which cannot only add the chromophore but also reduce the molecule soluble in water and add an improved affinity toward the fiber. Dyes may also be classified because of their solubility as soluble dyes, which include acid, mordant, metal complex, direct, basic, and reactive dyes; and insoluble dyes including azoic, sulfur, vat and disperse dyes (Dos Santos *et. al.*, 2007, Hunger, 2003, Yang *et. al.*, 2005).

Textile processing units, particularly wet processing units consume large quantities of water and therefore discharge wastewater in huge volumes. They also make use of various chemicals and dyes, which are harmful to the environment. The sources of effluent are: desizing, scouring, bleaching, mercerizing, dyeing,

Introduction

printing, washing and finishing. However the process to be followed varies in accordance with the characteristics required in the final product. Central Pollution Control Board has listed the dye and dye intermediate industry as one of the heavily polluting industries (CPCB, 1990). Textile processing generally uses very high salt concentration (especially NaCl, 40-100 g/l) for the efficient fixation of dyes onto fibres and therefore, textile wastewater have been found to contain high level of the salt (EPA, 1997).

A high alkalinity is due to the presence of elevated levels of bicarbonates and carbonates and hardness of the wastewater indicates the presence of calcium and magnesium carbonates. They contain residual dyes, mordants and auxiliary chemicals (acids, alkalis, nitrites, chromium salts, sodium chlorite, surfactants etc.). Effluents from printing units is primarily generated from final washing of printed fabrics contains gums, oils, unfixed dyes, resins, starch and soaps used in preparing dye paste and fixatives. Thickener such as Carboxy Methyl Cellulose (CMC), Starch, Polyvinyl Alcohol (PVA) make up the major fraction of wash waters.

Textile wastewater is a mixture of colorants (dyes and pigments) and various organic compounds used as cleaning solvents, plasticizers, etc. It also contains high concentrations of heavy metals, total dissolved solids, and has high chemical and biological oxygen demand. The major metal pollutants such as copper, zinc, chromium, etc. come mainly from the metal complex dyes and chromium salts used in wool dyeing or as oxidizing agents in sulphur dyeing. Their effluents are discharged either directly (mostly untreated/partially treated) or along with domestic wastewater. The application of such wastewaters to agricultural fields is quite common in rural India, which has led to biomagnifications of heavy metals in vegetables and cereals. Such as use of dye stuffs in textile paper, paint and printing industries and improper disposal of these stuffs into the water sources cause serious problem of pollution and health hazards due to presence of heavy metals above permissible limits.

In India with the high and intense demand for textile products, the textile mills and its waste water have been increasing proportionally and increasing water pollution. The amount and properties of discharged effluent vary from industry to

Introduction

industry depending on the water consumption and average daily product. Untreated or partially treated textile effluent is notoriously known to contain i) large amount of total dissolved suspended solids which increase the turbidity in water, ii) large amount of total dissolved solids limiting the industrial and, iii) high levels of chemical oxygen demand (indicating high degree of pollution) and biological oxygen demand thereby inhibiting aquatic habitats, iv) elevated temperatures which lower the rate of dissolution of atmospheric oxygen in the water. It also causes problems of foaming and color persistence, having a highly fluctuating pH affecting the solubility and chemical forms of most substances in water. Most of the heavy metals are essential for growth of organisms but are only required in low concentration.

It is also reported that textile and dyeing industry pose a major environmental threat because of the large amounts of water and dyes involved in the manufacturing process. Large amount of chemically different and employed for various industrial application including textile dyeing. Dyeing and printing industries generate waste mainly i.e. liquid effluents, solid wastes and air emission. However, liquid effluents are the most concern because of its high volume and pollution potential. Quantity, quality and nature of waste generated depend on the fabric being processed, chemical being used, technology being employed, operating practices etc. Incomplete usage of dyes and improper fixation results in the presence of color in waste water generated by the textile units. The dyestuff can be found as a suspension or in dissolved state in the effluent. The dyes are highly structured polymers and are very difficult to decompose biologically.

The major concern is the amount of waste water discharged from these units and the complex compounds and chemical load it carries which is disposed off on open lands and drains. The effluent of the textile industries contains bleaching agents, acids and alkalis, highly toxic dyes and different salts. Heavy metals like copper, cadmium, chromium, zinc, iron etc. are also found in textile effluents.

Dye consumption is maximum in Textile industry that was reported around 60% of the total dye production out of which 10-15 % of the dyes used for dyeing comes out as waste effluent. Printing clusters of Kaithun (Rajasthan) are uses huge

Introduction

volume of water for dyeing and printing process. After utilization, this water is discharged into the nearby water bodies/drains in its untreated/polluted form. Then the waste is carried to the main drainage system as a result the water quality becomes turbid. Besides this, effluent of these printing units also results in soil and land pollution due to disposal of fabric swatches, scraps and gums used in printing process. The residue of fabric swatches are also piled up in front of the dyeing and printing industries. The swatches since cannot be sold on weight are thrown on the streets which produces enormous odour also resulting in blockage of drains during rainy season.

Dyeing industry effluent is characterized by dark color, high EC, TDS, TSS, Total Hardness, BOD COD, low in suspended solids and nutrients such as nitrogen and phosphorus. Color varies in intensity and has blue and red dominant colors. Besides these, untreated dyeing effluent contains chemicals such as acetic acid, caustic soda, sodium hydrosulphate, mordants, reducing agents, soap and heavy metals such as copper, lead, nickel and cadmium. These heavy metals have a marked effect on aquatic flora and fauna through bio-magnification enter the food chain and ultimately affect human beings as well.

Dyeing effluents cause coloration of surface and ground water when released untreated/polluted thereby making it unfit for irrigation, drinking and cause severe problems to aquatic life. The World Bank estimates that 17-20 percent of industrial water pollution comes from textile dyeing industries. Out of the 72 toxic chemicals identified in dyeing industry effluent, 30 chemicals cannot be removed.

Dyeing and printing industrial wastes disposed both in liquid and solid forms in land and water bodies percolate into the groundwater and get transported in the direction of groundwater flow. The rate of percolation and transportation of pollutants in the groundwater flow direction increases in arid and semi-arid conditions due to high permeability of soil. As a result, different pollutants reach into the groundwater system and pose a threat to groundwater quality, which ultimately affects the socioeconomic life of the people, who depend on groundwater for various purposes.

Introduction

Looking into the content of waste water it can be measured of both positive and negative resources. The positive aspect of using waste water in agricultural activities is that it has nutrients which can be used for irrigation, thus benefits farming communities, societies and municipalities. The negative aspect of waste water reuse is the damaging effects on humans, animals and ecological system that need to be recognized and considered.

Irrigation with wastewater gives a short-period benefit to the farmers but it is not applicable for a long-period as it is hazardous for the soil and plants. Heavy metals like iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), lead (Pb), chromium (Cr), nickel (Ni), cadmium (Cd) and cobalt (Co) are present in the wastewater and accumulate in receiving soils. Most of the metals are retained in topsoil and their concentration decreases with increasing depth of the soil. These are extremely persistent in the environment; they are non-biodegradable and non-thermo-degradable and thus their accumulation readily reaches to toxic levels. It is reported that all over the world, nearly 20 million hectare of the land is being irrigated with wastewater, feeding approximately 10% of the world's population (Hamilton *et al.*, 2007). Several benefits and limitations are associated with wastewater irrigation these are highlighted as follows:

A. Benefits of waste water irrigation-

- Wastewater irrigation helps in production of crops and increases crop yields.
- It recycles organic matter and other nutrients in soil and can improve soil properties, fertility and texture.
- Nutrients enrichment in wastewater reduces the use of synthetic fertilizers and therefore reduces the cost of fertilizers.

The cost of irrigation from nearby channels is lower than the cost of ground water utilization.

B. Limitation of waste water irrigation –

- Long term irrigation increases the soil salinity and heavy metals contents.
- Waste water contains both pathogenic and non pathogenic microorganisms, which diseased the crops.
- Waste water irrigation reduces crop yields due to presence of high desirable components (nitrogen) and undesirable components.

1.2: Impact of Dyeing and printing effluent on Soil

Soil is an important system of the terrestrial ecosystem and it has been degraded and contaminated by various anthropogenic activities that change the physical, chemical and biological properties of soil; resulting in nutrient deficiency; soil toxicity, improper soil and crop management, alteration of agricultural lands and also human and animal health hazard through the food chain. Industrial discharge of untreated or partially treated effluents directly into surrounding agricultural lands is considered as the most significant anthropogenic activity responsible for soil pollution by various pollutants like heavy metals such as Cd, Cu, Zn, Cr, Ni, Pb and Mn. Agricultural crops can also be injured, ranged from visible marking on the foliage to premature death of the plants, when exposed to high concentrations of pollutants. Direct discharge of industrial effluents especially that without treatment may have deeply influence on physico-chemical and biological properties of soil related to soil fertility. A huge amount of effluent from textile mills is being discharged on land or into water courses. This effluent is characterized by high Biological oxygen demand (BOD), Chemical oxygen demand (COD), sodium and other dissolved solids as well as micronutrients and heavy metals. Whatever the pollution source may be, soil can act as a sink of heavy metals but three main kinds of ecological risks are associated to this fact:

The specifications for tolerance limit for the discharge of industrial effluents, prescribed by Bureau of Indian Standards (BIS), previously known as ISI are listed in Table 1.

Introduction

Table 1: Tolerance limits for discharge of Industrial Effluents prescribed by BIS

S. No.	Parameter	Industrial effluents discharged into inland surface waters	Industrial effluents discharged into public sewers	Industrial effluents discharged on land for irrigation	Industrial effluents discharged into marine coastal areas
1.	pH	5.5 to 9.0	5.5 to 9.0	5.5 to 9.0	5.5 to 9.0
2.	Temperature, $^{\circ}\text{C}$	< 40°C	45°C	--	45°C
3.	Total Dissolved Solids (mg/L)	2100	2100	1500	--
4.	BOD, (5 days at 20°C , mg/L (Max.))	30	350	100	100
5.	COD, mg/L (Max.)	250	--	250	250
6.	Calcium, mg/L (Max.)	--	--	300	--
7.	Magnesium, mg/L (Max.)	--	--	250	--
8.	Total Hardness mg/L	--	--	1000	--
9.	Oil and grease				

Table 2: Various Classes of Dyes

Name of the class	Description	Application
Direct	Direct dyes are applied in a natural or slightly alkaline dye bath, at or near boiling point, with the addition of either sodium chloride (NaCl) or sodium sulfate (Na ₂ SO ₄). They are also used as pH indicators and as biological stains.	cotton, paper, leather, wool, silk and nylon.
Acidic	Acid dyes are water-soluble anionic dyes that are applied to fibers using neutral to acid dye baths. Attachment to the fibre is attributed, at least partly to salt formation between anionic groups in the dyes and cationic groups in the fibre. Most synthetic food colors fall in this category.	Silk, wool, nylon and modified acrylic fibers.
Basic	Basic dyes are water-soluble cationic dyes. Usually acetic acid is added to the dye bath to help the uptake of the dye onto the fibre. Basic dyes are also used in the coloration of paper.	acrylic fibers, but find some use for wool and silk.
Mordant	Mordant dyes require a mordant, which improves the fastness of the dye against water, light and perspiration. The choice of mordant is very important as different mordant can change the final color significantly.	Wool, leather

Introduction

Vat	Vat dyes are essentially insoluble in water and incapable of dyeing fibre directly. However, reduction in alkaline liquor produces the water soluble alkali metal salt of the dye, which in this leuco form has an affinity for the textile fibre. Subsequent oxidation reforms the original insoluble dye.	Cotton, rayon, wool
Reactive	Reactive dyes utilize a chromophore attached to a substitute that is capable of directly reacting with the fibre substrate. The covalent bonds that attach reactive dye to natural fibres makes them among the most permanent of dyes.	Cotton, wool, silk, nylon.
Disperse	Disperse dyes are water insoluble that are finely ground in the presence of a dispersing agent and sold as a paste or spray-dried and sold as a powder. In some cases, a dyeing temperature of 130°C is required, and a pressurized dye bath is used.	polyester, polyamide, plastics
Sulfur	Sulfur dyes are two developed dyes used to dye cotton with dark colors. The initial bath imparts a yellow or pale chartreuse color, which is later treated with a sulfur compound in place that is required to be dark black.	cotton , rayon
Pigment	A pigment is a mixture of dye and an opacifying agent, such as white oxide powders which scatter light or dark colored powders that both absorb and scatter. So the colors they add are opaque. Most pigment used in manufacturing and the visual arts are dry colorants, usually ground into a fine powder. This powder is added to a vehicle (matrix), a relatively neutral or colorless material that acts as a binder. In contrast with dye, a pigment generally is insoluble and has no affinity for the substrate.	Pigments are used for paint, ink, plastic, fabric, cosmetics, food etc.

- The loss of productivity in the soil compartment,
- The pollution of ground water due to metal leaching, and
- The accumulation of pollutants in the food- chain, with effects on vegetation and animals, including humans.

1.3: Impact of dyeing and printing effluent on Crops

India is an agriculture based country and a major user of water resources for irrigation. But there is a great demand for water for irrigation while gallons and gallons of effluents are let out into water sources untreated. Industrial effluents containing heavy metals pose a threat to the ecosystem. The use of industrial waste water for irrigation of crops is a recent phenomenon and scientific community has focused its attention towards this only after 1940's when the problem of fresh water pollution due to waste water disposal became acute. Since the population is increasing at an alarming rate which has put tremendous pressure on natural resources such as land and water. To feed this growing population more and more land has to be put under cultivation which has in turn increased demand for irrigation water also. Industrial waste water can help in solving this issue to some extent as in addition to water it has got considerable amount of nutrients also.

Now a day, farmers are using these effluents for crop plants irrigation due to water scarcity, and found that the growth, yield and soil health get reduced. Crop plants respond adversely to higher concentrations of various industrial effluents depending on the source or type of pollutants and their toxicity level. When concentration of dyeing and printing effluent increase in irrigation water could also result in delayed flowering, fruiting and low yield, while another effect could be cytological and anatomical. Several other detrimental effects of higher concentrations of industrial effluents on germination and seedling growth of crop plants have been reported to be related to high heavy metal toxicity, which are hazardous and inhibit the functions of necessary enzymes.

Introduction

Using of industrial effluents or wastewater for irrigation has emerged in the recent past as an important way to utilizing waste water, taking the advantage of the presence of considerable quantities of N, P, K and Ca along with other essential nutrients. But there can be both beneficial and harmful effects of waste water irrigation on crops including vegetables and cereals. Therefore, it is highly required to study the impact of these effluents on crop system before they are recommended for irrigation. Effluent could be reused if concentration of all trace elements was found to be low and within the guidelines for irrigation of agricultural crops. Continuous use of wastewater leads to the enrichment of soil with essential macro and micro-nutrients.

Seed germination is an important and vulnerable stage in the life cycle of terrestrial angiosperms and determined seedling establishment and plant growth. Effluents discharged from the industries have either beneficial or lethal effects on the germination, growth and development of agricultural crops. The use of industrial effluent as alternative means of recycling in crop production is a common practice. The textile industrial effluents can have beneficial effects on the seedling growth and productivity of crops when they supplement soil fertility, organic matter and plant nutrient contents. Experiments have repeatedly demonstrated an increased productivity of crops or trees when irrigated with wastewater/effluent as compared with clean water. These nutrients represent a resource of considerable value when compared with the equivalent cost of fertilizer. The application of wastewater/effluent at rates which ensure a balance between nutrient input and plant uptake will promote optimal plant growth while limiting the risks of pollution (CSIRO, 1995).

1.4: Impact of dyeing and printing effluent on soil microbiology

Soil is an important natural resource formed as a result of millions of years of activity. It influences the distribution of plant species and provides a habitat for a wide range of microorganisms. Soil plays an important role in growth of plants and trees maintaining the ecosystem with its natural fauna and flora and indirectly sustains the environment. The effluent from the industries contains large number of bacteria and fungi. Some of these microorganisms are beneficial to the plants. These organisms help to breakdown the complex organic matter into the simple

Introduction

form and consequently increase the fertility of the land. But some micro-organisms are pathogenic for the plants. These organisms can cause different diseases to the plants. The effluent also contains different chemicals in high amount. These chemicals in high amount serve as hazardous to the plants. The industries utilize many toxic substances which are very harmful to the plants and soil microorganisms. Soil degradation from inorganic and organic contaminants is not only ecological risk but simultaneously is a socio economic issue; soil becomes poor in physicochemical properties susceptible to erosion, loss of productivity, sustainability and diminished food chain quality. Healthy soil has microorganisms essential for the decay and decomposition of dead organic matter and provides the fertile layer humus essential for plant growth. The health of soil is indicated by diversity of micro flora. Microbial population of these soils showed difference with the micro flora of normal soil. This could be attributed to many facts like availability of organic nutrients, nitrogen and other minerals, increase in acidity due to the dyes specially azo dyes leads to oxygen depletion.

The soil was made barren by the textile effluents and even could not support the growth of grasses. Various pollutants (both organic and inorganic) present in the wastewater/effluent affect the indigenous microorganisms by decreasing their number, reducing their biochemical activities, affecting diversity and changing community structure. However, continuous exposure of various toxic chemicals on microorganisms also helps in the development of a resistant microbial populations having the mechanism of degrading or even utilizing the organic pollutants.

Soil contains various types of microorganism including bacteria, which are the most important and abundant microorganism which is present in surrounding environment. These are very small, unicellular, primitive and non chlorophyll containing microorganisms. Among the soil bacteria a unique group called *Rhizobia* has a beneficial effect on the growth of plants. It can live either in the soil or within the root nodules of host legumes. Legume plant posses a unique ability to establish symbiosis with nitrogen fixing bacteria of the family *Rhizobiaceae*. *Rhizobia* are the name given to the group of genera of alpha-proteobacteria (family *Rhizobiaceae*) which includes all of the nitrogen fixing species that produce nodules with legumes.

Introduction

This symbiotic relationship reduces the requirements for nitrogenous fertilizers during the growth of leguminous crops and also enriches soil with nitrogen. *Rhizobia* and their legume host must recognize each other for nodulation to begin. Though legume *Rhizobium* symbiosis has been extensively studied in many crops but no systematic work has been done to exploit the nodulation and nitrogen fixing ability of these legume crops for increasing the production and impact of dyeing and printing effluent on germination, ecomorphological and biochemical characters of selected legume plants and also the morphological, cultural and biochemical characterization of *rhizobium*.

Rhizobium is able to enter into symbiotic relationship with legumes. They fix atmosphere nitrogen and thus not only increase the production of the inoculated crops, but also leave a fair amount of nitrogen in the soil, which benefits the leguminous crop plants. Following groups of *rhizobium* have been recognized for inoculating legumes in India. *R. leguminosarum*, *R. meliloti*, *R. trifoli*, *R. phaseoli*, *R. lupini*, *R. japonicous* etc. *Rhizobium* as a bacteria are a tiny and lower most components of any food chain, but these tiny members have their own importance, without these *rhizobium* bacteria we can't imagine legumes and nitrogen cycle in atmosphere. So it is very important especially in area where large amount of soil and air polluted, by various pollutants such as effluent, which is directly dumped in soil. Direct and indirect dumping of effluent may alter the characters of leguminous plants and bacteria present in soil and nodule of those plants.

Among plant-microbe interactions, legume-rhizobium interactions are unique because they supply 80-90% of total nitrogen requirement of legumes. It involves a complex interaction among host, microbial symbiont and environment. Among nitrogen fixing systems, legume-rhizobium symbiosis is one of the most promising and the bacterial species of Rhizobium complex are very important.

Pink or red nodules should predominate on a legume in the middle of the growing season. If grey or green nodules predominate, little nitrogen fixation is occurring as a result of an inefficient rhizobium strain it may be due to poor plant nutrition, pod filling or other plant stress. Any stress that reduces plant activity will reduce nitrogen fixation. It indicates that if there is any alteration in character of

Introduction

soil, the root nodules may affected positively or negatively. Factors like temperature and water may not be under the farmer control. But nutrition stress especially phosphorus, potassium, zinc, iron, molybdenum and cobalt can be controlled by improving soil quality. When a nutritional stress is corrected, the legume responds directly to the nutrient and indirectly to the increased nitrogen nutrition resulting from enhanced nitrogen fixation. Poor nitrogen fixation in the field can easily be corrected by inoculation, fertilization, irrigation or other management practices. It is very important for good growth of plant (leguminous) the soil should not be contaminated.

Legumes have been suggested as appropriate crops for the enhancement of bio productivity and improvement of marginal lands, because these plants not only yield good fodder, protein rich seeds and fruits, but they also enrich soil nitrogen in symbiotic association with *Rhizobium*. Legume plant may utilize wastewater effluent and uptake heavy metals through extensive root system. Thus, these plants serve as effective biological sieves, inhibiting contamination of ground water sources. Nodulation and nitrogen fixation in legume- *Rhizobium* association are adversely affected by water quality can preclude legume establishment and growth, or reduce crop yield. Unsuccessful symbiosis may be due to failure in the infection process because of the effect of water quality on the establishment of *Rhizobia*. Legumes and the process of nodule initiation are both more sensitive to osmotic stress than any other rhizobium.

It is well known fact that in the present context there is a limited availability of organic manure in agriculture due to population explosion, intensive agriculture, reduction in livestock population etc. Therefore, the judicious application of nutrients is essential to keep the soil fertile and to make the agriculture sustainable. This calls for the use of alternate sources of nutrients in agriculture. Irrigating industrial effluent provides farmers with a nutrient enriched water supply and society with a reliable and inexpensive system for wastewater treatment and disposal.

In the present investigation an attempt has been made to assess the impact of dyeing and printing effluent released from a local dyeing industry at Kaithun

Introduction

reagion, Kota. Effluents discharged from the industries have either beneficial or lethal effects on the germination, growth and development of agricultural crops. The beneficial and harmful effects of the different concentration of effluent on crops have been assessed and after suitable dilution can be used as liquid fertilizer for several crops. Present research work deals with the physico-chemical properties of dyeing and printing effluent and their effect on leguminous crop plants (*Glycine max L.* and *Medicago sativa L.*) treated with different concentrations of effluent. The analysis work on growth and yield of crop plants is extremely rare thus, the current research work deals with the germination, growth, yield, biochemistry and nodulation study of *Glycine max L.* and *Medicago sativa L.* at different dilution to find out the effects of dyeing and printing effluents.

1.5: Main objectives of present research work are -

1. Analyze the physico-chemical characteristic of dyeing and printing effluent collected from experimental site.
2. Analyze the physico-chemical characteristic of soil of experimental site.
3. Identification and characterization of *Rhizobial* strains isolated from effluent contaminated soil.
4. Laboratory experiments will be conducted to find out the effect of dyeing and printing effluent on *Rhizobial* strains and nodulation of *Glycine max L.* and *Medicago sativa L.* plants by using Pot culture method.
5. Statistical analysis of some growth parameters of experimental plants.
Following parameters will be taken-
 - Length of shoots and roots, fresh and dry weight of shoot and root
 - Productivity (Pods/Plant, Pods weight, Seeds/Plant, Seeds weight)
 - Nodulation study (Nodules/plant, fresh and dry weight of nodules/plant).

References:

1. Bureau of Indian Standards Drinking Water Specification IS: 10500.
2. Central Pollution Control Board (CPCB).
3. Climate Change Research Program (CSIRO).
4. Dos Santos, B., F. J., Cervantes, and J.B., Van Lier.
5. Environmental Protection Agency (EPA).
6. Hamilton, A.J., F., Stagnitti, X., Xiong, S.L., Kreidl, K.K., Benke, P., Maher.
7. Hunger K.
8. Yang, C., M.C., Jared, and J. Garrahan.

1.6 Study Area:

Textile industries are large industrial consumers of water as well as producers of wastewaters with the increased demand for textile products. However surface water is also being polluted by anthropogenic activities and idol immersion. The textile industries and its wastewaters have been increasing proportionally, making it one of the main sources of severe pollution problems worldwide. The important pollutants in textile effluents are chiefly recalcitrant, organic, colors and toxicants. Large number of dyeing and printing industries are located in the various districts of Rajasthan viz Barmer, Jaipur, Sikar, Jodhpur, Bikaner, Pali, Chittaurgarh, Udaipur, Sanganer, Bagru, Bassi, Jairmapura, Kota (Kaithun).

Kota Doria is made in many villages located in Kota, Bundi and Baran districts of Rajasthan. However, the oldest and biggest concentration of weavers is in Kaithun, situated about 18 kms towards South from Kota (the District Headquarter). Kaithun is a town and a municipality in Kota district in the Indian state of Rajasthan. In Kaithun, there is significantly more production of Kota Doria,

Introduction

with some 2700 weavers engaged in the sector. According to Census 2011 information the location code or village code of Ladbura Kaithoon village is 102053. Ladbura Kaithoon village is located in Ladbura Tehsil of Kota district in Rajasthan, India. It is situated 18km away from sub-district headquarter Ladbura and 18km away from district headquarter Kota. As per 2009 stats, Jakhora is the gram panchayat of Ladbura Kaithoon village.

The total geographical area of village is 301.67 hectares. Ladbura Kaithoon has a total population of 1,973 peoples. There are about 379 houses in Ladbura Kaithoon village. Kota is nearest town to Ladbura Kaithoon which is approximately 18km away. Kaithun is surrounded by Kota Tehsil towards South, Sultanpur Tehsil towards East, Sangod Tehsil towards West, and Keshoraipatan Tehsil towards North.

Table 3: Ladbura Kaithoon- Village Overview

Village	Ladbura Kaithoon
Gram Panchayat :	Jakhora
Block / Tehsil :	Ladbura
District :	Kota
State :	Rajasthan
Pincode :	325001
Area :	301.67 hectares
Population :	1,973
Households :	379
Nearest Town :	Kota (18 km)

A. Selected Study Site

Kaithun is a rural Indian town located in the 25.14070N and 75.96790E of Kota region at a distance of about 14 kilometers from Kota city. Kaithun has a unique place in textile industry resource. It is known for natural dyes and hand block printing. The town had a community of chippas who print fabrics by hand. Kaithun prints are more famous for their exceptional quality of being eco-friendly and forms the most essential part of the block printing industry of Rajasthan. Thus, printing in this region is mainly carried out by the natural dyes but some enterprises make use of synthetic dyes including VAT and AZO dyes and mordents. Mordents are used for fixing of colors and contain heavy metals which are toxic in nature.

The colored effluents of various cluster units (dyeing and printing units) of Kaithun have got much attention to their dual toxicity. Also the effluent from these units and industries were discharged in open land, agricultural land, thus causing an adverse effect on flora, fauna and general health of residents residing in the communities within around the sides and town downstream.

Kaithun is a small town in the Kota district where Kota Doria is mainly woven. Some clusters of Kota Doriya can also be found in villages of Bundi, Baran and Kota, but Kaithun remains the major hub of its production. The weaving of Kota Doriya is a household activity. At least one pit loom for weaving can be found in almost every house in Kaithun. Though all members of the family participate in the process, it is mainly handled by the women of the house.

Weaving process:

Windind: Winding is the process of transferring yarn from these hanks on to bobbin for wrap and on pirn for weft.

Warping: Warping is the process of obtaining a predetermined length of wrap having desired number of threads that shall be needed for the complete width of the fabric.

Dyeing: The yarns are dyed (or colored) manually by dipping them in utensils filled with heated water and pre dissolved dyes.

Introduction

Sizing: Sizing is done in order to impart strength to the yarn and is required mainly in cotton. A thin rice paste (maandi) and juice of locally available wild onion is used for this purpose.

Drafting-Denting-Piecing: All individual cotton and silk threads are drafted through double clasped country cotton heald (Ranch) and dented through dents of bamboo/ steel reed (Fani) in a particular pattern to produce the checks or ‘Khat’ along with the weft. Since drafting and denting of individual threads is time-consuming, an alternative method called piecing is used in which the individual new threads of the wrap are tied to the corresponding threads of the previous leftover warp.

Weaving: Weaving is done on the pit looms by throw shuttle technique. It is done so skillfully that almost uniform size of the ‘khat’ or the check pattern is produced.

Designing: The designs to be produced are transferred to the fabric through various techniques.

B. Sampling sites

The effluent was collected from a small scale dyeing unit at kaithun of Kota, Rajasthan. Kaithun is famous worldwide for its handloom textile products. The samples were collected in pre-cleaned 5 Liter polythene bottles from the point of discharge of the effluent from the industry and preserved in a refrigerator at 4°C till the completion of the investigation.

Table 4: List of Sampling Sites

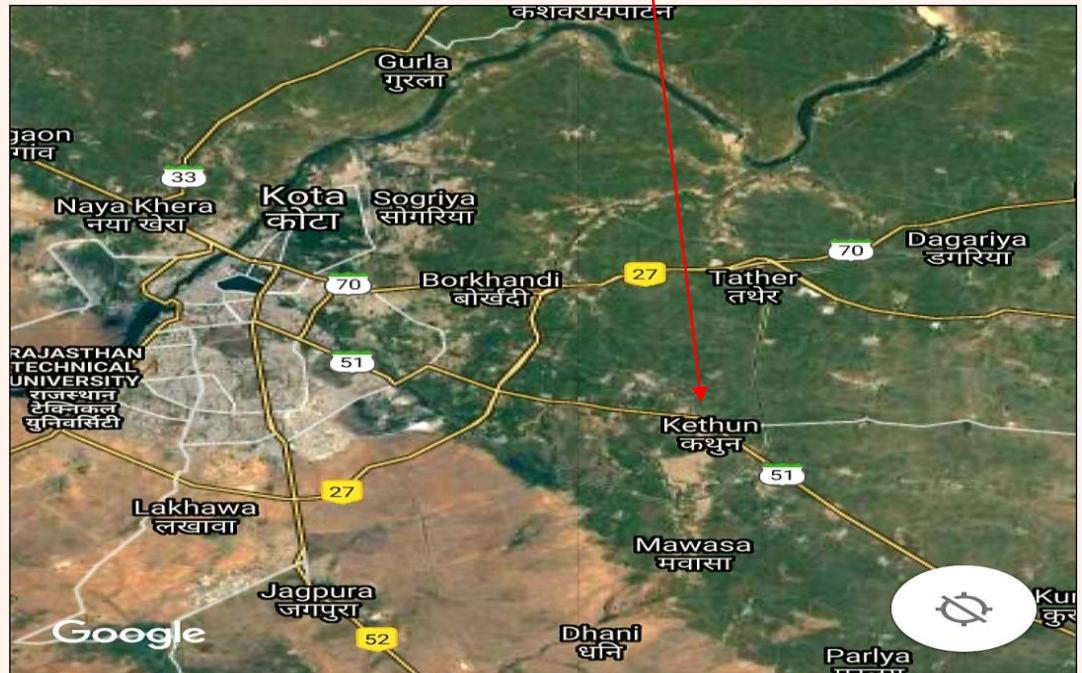
S. No.	Sample No.	Sampling Site
1.	S1	Bhimpura outside kaithun STM
2.	S2	Ahirwada middle area of kaithun STM

- STM – Small Textile Mill

PLATE - 1



(A) Study site in Rajasthan Map



(B) Study site in Google Map

Experimental site

PLATE - 2



(A) Weaving Process



Source:Utsavpedia.com

(B) Weaving Process

Weaving Process

PLATE - 3



(A) The dyed fabric is then stamp printed (Site 1)



(B) The dyed fabric is then stamp printed (Site 2)

Printing Process

PLATE - 4



(A) Effluent discharge (site 1)



(B) Effluent discharge (site 2)

Various Effluent Discharge Site

PLATE - 5



(A) Preparation of Paint for Printing process



(B) Preparation of Binders and Fixers



(C) Dyeing Process

Preparation for Printing Process

CHAPTER – 2



REVIEW OF LITERATURE

REVIEW OF LITERATURE

The aim of the present study was to investigate the impact of dyeing and printing industry effluent on the growth of leguminous crops. Industrial effluents discharged from the dyeing and printing industries contain higher amount of pollutants including heavy metals. These effluents released on the land and surface water ultimately leach into the ground water, contaminate it and lead to a series of problems in living beings because of their non-biodegradable nature. Hence, the environmental problems and health hazards posed by dyeing and printing industry effluents are becoming more complex and critical.

On the other hand, there is an increasing interest in the agricultural use of these industrial effluents, because of the wide scope for using these as nutrient sources and irrigation water. Moreover, the application of industrial effluents for irrigation reduces the water scarcity to a large extent. Hence, reuse of industrial waste water in agriculture is an alternative strategy of eco-friendly disposal, which offers the benefit of reducing pollution, water management, besides improving the productivity of crops. The literature reviewed here pertains to physico-chemical parameters of industrial effluents, impact of industry effluents on growth, biochemical characteristics, yield performance, nodulation and soil microbiology of crop plants are critically reviewed in this chapter under different heading.

2.1: Physico-chemical character of textile wastewater

The industrial effluents, which are now increasing used as source of essential nutrients and/or as a source of irrigation, also contains different pollutants and thus are a potential threat for pollution of soil and ground water. Effluents are liquid waste materials that are by-products of human activities such as liquid industrial discharge.

Untreated or incompletely treated industrial wastewater contains chemical constituents such as calcium, magnesium, sodium, potassium which are the major cations and anions like chloride, nitrate, sulfate and heavy metals. These chemical constituents are measured in terms of various physico-chemical parameters such as

Review of Literature

pH, EC, total dissolved solids, total hardness, and total alkalinity. The physico-chemical parameters of the effluent while being discharged must be within the permissible limit proposed by BIS and if goes beyond then it causes environmental pollution. Heavy metals such as mercury, lead, cadmium, chromium, nickel and zinc tend to accumulate in living tissue and proceed along the food chain. The characterization of the waste water produced by any industry is very important in order to know the quantity and quality of constituents present in it and based on which its safe use in agriculture can be planned to recycle waste water, increase productivity and maintain soil health.

Ajmal and Khan (1985) found textile effluent brown in color with low dissolved oxygen, high values of TDS, TSS, BOD and COD, collected from Modi Textile Factory Ltd., Modinagar, UP. Phosphorus (17 mg/l), chlorides (780 mg/l), sulphates (400 mg/l), sodium (195.4 mg/l), calcium (280 mg/l) and magnesium (140 mg/l) were also found in a significant amount.

Gupta and Jain (1992) analyzed textile industry effluent releasing from hand processing units of Pali (Rajasthan) with high salinity (SAR: 82), BOD (400-800 mg/l), COD (900-1500 mg/l), excessive concentrations of sodium and carbonate ions (RAS: 42 mg/l), high alkalinity (pH: 10-11.5) and unduly low concentrations of calcium.

Rao *et. al.* (1993) analyses the textile industry chemical properties. The pH Varied from 5.02 to 11.0. EC was found to be from 7.5 ms/cm to 1.79 mc/cm. BOD level of untreated textile effluent ranged from 407 mg per liter to 662 mg/l which was higher than the permissible limit (30 mg/l) of central pollution control board (CPCB). COD of untreated textile effluents varied from 780 mg/l to 1460 mg/l and values of the COD beyond the permissible limit (250 mg/l) of CPCB. So, increase in BOD and COD of textile effluent may cause hypoxia conditions with consequent adverse effects on aquatic biota.

Banat *et. al.* (1996) reported that the physico-chemical nature of the effluents vary depending upon the different type of material used during the textile production coloring of wastewater is mainly due to the rains of dyes and the coloring agents.

Review of Literature

Balakrishnan *et. al.* (2008) worked on Impact of dyeing industrial effluents on the groundwater quality in Kancheepuram (India) and 20 groundwater samples were collected from various parts of the dyeing industrial region and the samples were analyzed with standard analytic methods. The concentrations of total dissolved solids (1138 to 2574 mg/l), chloride (216 to 847 mg/l), total hardness (225 to 760 mg/l), sulphate (64 to 536 mg/l), nitrate (up to 58 mg/l), iron (up to 2.3 mg/l) and lead (up to 0.281 mg/l) were found to be higher and exceeded the permissible limits of BIS and WHO standards.

Deepali *et. al.* (2011) carried out comparative study on textile and tannery industry effluent at Haridwar in order to assess the metal analysis of the two selected industries testified higher amount of chromium from the permissible limits 1mg/l of central pollution control board (CPCB 2001). Treatments of the effluents resulted in lowering the levels of metals in treated effluent like Cu, Cr, Fe and Mn. Levels of the metals decreased upto 78.4, 66.6, 39.8 and 100 percent respectively.

Kaur *et. al.* (2010) analysed the physico-chemical characteristics of different textile effluents collected from panipat . They reported that BOD 181 to 306 mg/l and COD were in the range of 181 to 306 mg/l and 3853 to 4691 mg/l respectively, whereas pH ranged from 8.1 to 9.1. The values of total suspended solids (TSS) and total dissolved solids (TDS) were 190 to 163 mg/l and 4354 to 5768 mg/l respectively.

Verma and Sharma (2011) worked on analysis of physical and chemical parameters of textile waste water collected from Alwar and reported that textile waste water was basic (pH-8.3). BOD (350 mg/l) and COD (770 mg/l) levels were high. TDS (2352 mg/l) and TSS (270 mg/l) levels was exceed the permissible limits. Sodium (520 mg/l) level was quite high in textile effluent.

Islam *et al.* (2011) revealed that the dyes in surface and subsurface water is responsible for surface water and underground water and causes many water born diseases. For example, irritation of respiratory tract, mucous membrane and dermatitis.

Review of Literature

Esabela (2011) revealed physico-chemical properties of untreated irrigation water from amanishah nalla, Sanganer, (Jaipur). Water samples collected from the agricultural fields of *Trigonella foenumgraecum* grown in Sanganer area. The waste water was alkaline (8.4 -11.9) in nature, TDS ranged between 750 - 4670 mg/l and Chlorides from 315.40 to 1304.60 mg/l. Heavy metal concentration in waste water was quite high, ranged from 1.998 to 4.656 mg/l (Zn), 1.165 to 6.118 mg/l (Cu), 1.967-3.136 mg/l (Cr), and 1.106 to 3.949 mg/l (Pb).

Ahmad *et. al.* (2012) studied on effect of dye industrial effluent on physico chemical characteristics of soil at Bhairavgarh, Ujjain, and reported that water was alkaline in nature and EC was 410.38-500.46 $\mu\text{S}/\text{cm}$. Chloride concentration was highest (700mg/l) in sample 1 and lowest (500mg/l) in sample 2. The values of calcium (328.5-382 mg/l), magnesium (27.16-121 mg/l), sodium (63.5-70.2 mg/l) and potassium (36.3-39.7 mg/l) were high. Results of soil samples indicate its neutral to slight alkaline in nature and conductivity was 373-418 $\mu\text{S}/\text{cm}$. Other parameters like water holding capacity (60-65%), organic carbon (0.32-0.42%) and organic matter (0.55-0.72%), calcium (189-273 mg/kg), magnesium (8.50-45.9 mg/kg), sodium (70.3-75.7 ppm), potassium (50.7-58.7 ppm) and phosphorous (0.83-0.98 mg/kg) were also shows a wide range in polluted soil samples as compared to unpolluted samples.

Kibria *et. al.* (2012) evaluate the effects of wastewater irrigation on the heavy metals accumulation in soils and their uptake by plants. Samples were collected from sixteen wastewater-irrigated sites from four locations in Chittagong city, Bangladesh. The heavy metals (Cd, Pb, Zn, Cu and Mn) in top soil zone and concentration of these heavy metals in plants were analyzed. Bioaccumulation coefficient of Cd, Pb, Zn, Cu and Mn in plants ranged from 0.20 ppm to 13.91, 0.008 to 0.72, 0.006 to 1.60, 0.03 to 0.64 and 0.01 to 0.73 ppm respectively.

Joshi *et. al.* (2012) analyzed the waste water from Sumukh textile mill at Vapi, Gujarat. The physico-chemical properties and heavy metals concentration were also analyzed for the study. They reported that waste water was blackish brown in color with strongly sour smell. The COD (2078.55mg/l) and TDS (3850.23mg/l) values were very high.

Review of Literature

Thoker *et. al.* (2012) worked on Impact of Dye Industrial Effluent on Physicochemical Characteristics of Kshipra River, Ujjain City, India and reported that high levels of COD (73-345 mg/l), pH (7.6-9), TS (2100-6050 mg/l), TDS (1990-5820 mg/l), DO (0-8 mg/l), total hardness (321-880 mg/l) were observed, which exceeds the standard levels of BIS and world health organization (WHO).

Paul *et. al.* (2012) selected six textile industries in east region of Solapur city for analyzing the major pollution indicator parameters i.e. BOD, COD, TDS, sulphide, sulphate, chloride, hardness, alkalinity, calcium and magnesium. They found that COD (1548 ppm), TDS (7072 ppm), sulfide (79 ppm) , chloride (2750 ppm) and sulphate (912 ppm) respectively.

Goyal *et. al.* (2013) collected the textile water samples from twenty different locations in bagru region Jaipur and revealed that a large variation in the physico-chemical characteristics of textile effluents. The pH and electrical conductivity were in the range 5.40 to 10.34 and 2.19s/cm to 5.85s/cm respectively. BOD (221 mg/l - 699 mg/l) and COD (1170 mg/l - 3998 mg/l) values were found higher than maximum permissible limits (WHO) in all the samples.

Jaishree and Khan (2013) analysed the physico-chemical characteristics of the textile effluent contaminated soils and found a large variation. The pH of the different textile effluents was ranging from 7.8 to 9.4 and electrical conductivity was in the range of 0.75 to 1.15 mmhos/cm. The organic carbon ranged from 0.24-0.42%.

Mathur and Kumar (2013) evaluated the physico-chemical characterization of industrial effluents contaminated soil of sanganer and reported that soil samples were highly colored, foul smelling and alkaline (pH 8.8) and contaminated trace metal ions with concentrations values which were not in compliance with standards. The pH of the samples was alkaline in nature (8.0-8.8). The values of electrical conductivity ranged from 0.19%-0.81% mmhos/cm. The amount of % organic matter and % organic carbon ranged from 0.31-0.41% and 0.18-0.24% and available phosphorus (29 to 36 kg/ha) and potassium (190-310 kg/ha) respectively.

Sharma *et. al.* (2014) investigate comparative analysis of physico-chemical characteristics of dyeing industry effluent between sanganer and bagru printing

Review of Literature

clusters, Jaipur and reported that physicochemical properties such as BOD (390-435 mg/l), COD (250-1650 mg/l), pH (6.7-8.9), and EC (1.42-4.16 ms/cm) of bagru and BOD (400-425mg/l), COD (250-2500 mg/l), pH (6.9-9.5), and EC (3.5-4.5 ms/cm) for sanganer exceed from prescribe limit by rajasthan state pollution control board (RPCB). Ghaly et al. (2014) revealed that the effluent released from the textile printing and dyeing process was highly polluted with high BOD and COD values. Besides this, slightly alkaline in nature and contain heavy metals.

Kanan *et. al.* (2014) depicted the effects of textile effluent on soil and water. The physico-chemical parameters of water and soil were also analyzed and found that pH ranged from 7.56 to 9.68, EC ranged from 17040 to 38040 $\mu\text{s}/\text{cm}$, COD ranged from 810.60 to 1430.43mg/l and BOD ranged from 305.58 to 608.16 mg/l . The amount of N and temperature varied from 0.217 to 0.787 $\mu\text{g/g}$ and 30.24 to 51.22°C respectively.

Karim *et. al.* (2015) worked on physico-chemical and microbiological analysis of textile dyeing effluents in Chittagong city and reported that pH of the collected samples was ranged from 7.6 to 8.56 while temperature in the range of 30°C to 37°C . BOD, COD, TSS and TDS values were found to be 220 mg/l, 342 mg/l, and 900 mg/l, 2933 mg/l for inlet and 120 mg/l, 214 mg/l, 800 mg/l, 1766 mg/l for discharged effluent respectively.

Rashmita *et. al.* (2015) described the physico-chemical analysis of textile effluent collected from South Gujrat region and found that pH (4-13), Dissolved oxygen (6-12 mg/l), BOD (72-93 mg/l), COD (32-58 mg/l), chloride (180-289 mg/l) were varied with BIS standard. Mobar *et. al.* (2015) studied soil samples from agricultural field receiving textile effluent in Sanganer area and an untreated agricultural field of Durgapura area and found high values of pH, EC, water holding capacity, total hardness and sodium values.

Sriram and Reetha (2015) evaluated the physico-chemical characteristics of textile dye effluent and reported that pH (7.9), electrical conductivity ($223\mu\text{S}/\text{cm}$), chemical oxygen demand (763 mg/l), biological oxygen demand (178 mg/l), dissolved oxygen were (115 mg/l) respectively. They also reported that chloride (1124 mg/ml) is found in high amount in the textile dye effluent.

Review of Literature

Manikandan *et. al.* (2015) characterized the textile effluent of industry of Tirupur city, India for various parameters like COD, BOD, TDS, alkalinity, pH, total hardness, sulfate and chloride. The effluent was highly turbid and colored. The BOD/COD ratios ranged from 0.2 to 0.5 which indicated that the effluent contained a large proportion of non-biodegradable organic matter. The effluent had high concentration of sulfate, chloride, calcium and magnesium, which are responsible for higher total hardness of effluent.

Mishra and Soni (2016) worked on analysis of dyeing and printing waste water of Balotra textile industries and found to contain pH and electrical conductivity in the range 7.1 to 8.7 and EC from 2000 to 9000 $\mu\text{mhos}/\text{cm}$, TDS (467-18040 ppm) and COD (150-1289 ppm respectively.

Elango *et. al.* (2017) depicted the physico-chemical parameters of textile dyeing effluent, Tamilnadu and its impacts and concluded that pH (8.6), total hardness (970 mg/l), BOD (970 mg/l), COD (3080 mg/l), TDS (242220 mg/l), TSS (7116 mg/l), turbidity (81.5 NTU), chloride (42487 mg/l), silica (1087 mg/l), oil and grease (18 mg/l) of the effluent were above guideline permissible limits.

Malik (2017) worked physico-chemical and microbial analysis of the soil contaminated by textile industries located in sanganer industrial area, jaipur and reported that pH (7.72-9.6), EC (0.66-3.38mS/ μS), organic carbon (0.15%-0.92%), alkalinity (4-50 mg/l) and microbiological parameters of soil sample showed bacterial colony count in textile industry was very high (79×10^6 - 120×10^6) as compared to forest soil 'A' (44.33×10^6 - 89×10^6) which shows that large amount of microorganisms were taking part in the degradation of industrial effluents.

Sathiyaraj *et. al.* (2017) examined the physicochemical characteristics of textile effluent collected from Erode, Pallipalayam, and Bhavani polluted regions, Tamil nadu and reported that irrespective of the source, effluent properties such as pH (8.75, 7.51, 7.42), total dissolved solids (6040 mg/l, 5327 mg/l, 5131 mg/l), chemical oxygen demand (859 mg/l, 802 mg/l, 609 mg/l), biological oxygen demand (450 mg/l, 340mg/l, 260 mg/l), sodium chloride (2125 mg/l, 2050 mg/l, 1725 mg/l), calcium (134 mg/l, 120 mg/l, 104 mg/l), magnesium (19 mg/l, 17 mg/l,

16 mg/l), potassium (95 mg/l, 80 mg/l, 65 mg/l) and heavy metals exceeded permissible limits by the WHO/Food and Agriculture Organization/Federal Environmental Protection Agency for irrigation.

2.2: Effect of textile industry waste water on soil microbial population

Soil is a complex environment offering a variety of microorganisms. The microbial community is one of the most important components of soil. This is one reason why microbial diversity in soils is much greater than that found in any other environment. Microbial diversity depends on available nutrients and their varied concentrations. These microorganisms decompose organic matter and play important role in various biological process taking place in the soil. The discharge of polluted/untreated textile effluent in the soil affects the soil health and its productivity. The soil micro-organisms are the major constituents of soil ecosystem involved in the recycling of nutrients, whose activity gets affected due to textile industry waste water application. Since the soil health is being measured through its microbial population, it becomes essential to know the impact of textile effluent on soil micro flora. When this untreated/polluted effluent is permitted to run in the fields, it obstructs the pores of the soil causing in loss of soil fertility and yield. The consistency of soil is toughened and permeation of roots is prohibited. Now a day's effluent from textile dyeing industries is unsafe for soil.

Ajmal and Khan (1985) reported that even application of textile effluent on short term basis in soil results in increase of water soluble salts, organic matter, nitrogen and phosphorous content of soil. Some small short field trials have also reported the deleterious effects of textile effluents on soil health.

Baath (1989) reported that Microbial growth in the soil is fostered by the presence of certain metallic compounds when available in a considerable amount. Excess amount of sodium present in the soil; bind the metal ions which therefore cannot be taken up by the microorganisms resulting in degraded growth of the microorganisms. Chander and Brookes (1991) reported that low microbial biomass and activity may limit the decomposition of soil organic matter and lead to the accumulation of organic materials in metal-contaminated soils.

Review of Literature

Sarnaik and Kanekar (1995) isolated the four species of *Pseudomonas*, namely *P. alcaligenes*, *P. mendocina*, *P. putida biovar B* and *P. stutzeri*, from the soil samples in the premises of the factory manufacturing methyl violet, nearby Pune. There was an alteration and a reduction in number of *Pseudomonas* species from soil samples collected from premises of a dye factory.

Aoyama *et. al.* (1997) studied that Heavy metals at high concentrations may have great impacts on soil microbial community structure, biomass, and activities. The adverse effect of heavy metals has often been observed as a reduction in microbial biomass and activity. Chandy (1999) tested the inhibitory effect of sixteen heavy metals on bacteria at five different concentrations and found a gradual decline in their growth with the increasing concentration of heavy metals. Moreover, several workers have also reported that heavy metals introduced into the water not only bring about several chemical changes and high degree of variation in metal concentration but also affect the entire ecosystem including the bacterial population

Kuske *et. al.* (2002) reported that fertility and productivity of the soil is hampered by the textile dye effluent when used for irrigation purposes. Water holding capacity is severely degraded at the sites contaminated by the chemicals and metals because of a change in the bacterial and fungal population growth due to the presence of chemicals and metals.

Shi *et. al.* (2002) observed that reduced microbial activity may originate from the change of microbial community structure after long-term exposure to heavy metal pollution. Heavy metals are often mixed with organic pollutants in contaminated sites. The presence of multiple contaminants may present extreme challenges to the maintenance of a phylogenetically and functionally diverse microbial community. In soils contaminated with both heavy metals and hydrocarbons, only microbes that tolerate both heavy metals and toxic levels of hydrocarbons may survive.

Nicholson *et. al.* (2003) studied that the soil is a long-term sink for the group of potentially toxic elements often referred to as heavy metals like zinc, copper, nickel, lead, chromium and cadmium. Whilst these elements display a

Review of Literature

range of properties in agricultural soil including differences in mobility and bioavailability, leaching losses and plant uptake are usually relatively small compared to the total quantities entering the soil from different diffuse and agricultural sources. As a consequence these potentially toxic elements slowly accumulate in the soil profile over long periods of time. This could have long-term implication for the quality of agricultural soils, including phytotoxicity at high concentrations, the maintenance of soil microbial processes and the transfer of zootoxic elements to the human diet from increased crop uptake or soil ingestion by the grazing livestock.

Faryal *et. al.* (2007) identified Microbial community in the soil samples of textile mill effluents in Rawalpindi was found to be different from the reference soil (the uncultivated land 5 kms away from the textile mills). The study clearly demonstrated the changes experienced in the physiochemical parameters of soil on the contaminated site to that of the reference site. Findings suggested on the monitoring of the effect of waste water used for irrigation purposes. Three genera identified on the effluent irrigated soil were *Bacillus*, *Micrococcus* and *Listeria*. Metals like Chromium, Zinc, and Copper were found to be above permissible limits of the contaminated site.

Stefanowicz *et. al.* (2008) studied the effects of metal contamination on soil bacterial and fungal functional diversity and reported that with increasing metal concentration, bacterial functional diversity decreased significantly. Kaur *et. al.* (2010) studied the Characterization of microbial community in the water bodies and soil systems receiving the textile wastewater would provide valuable information about the effects of the effluents on the ecosystem health. Soil represents the most favorable habitat for variety of microbes like bacteria, fungi, algae, viruses and protozoa, but unfortunately becoming a sink for various industrial effluents from several decades. They analyzed the physico-chemical parameters of industrial effluents (sugar and textile industry) and their impact on soil microbial flora. They have observed that the effluents caused reduction in total bacterial counts.

Prasad and Rao (2010) isolated *Bacillus sp.*, *Klebsiella sp.*, *Salmonella sp.* and *Pseudomonas sp.* from the textile effluent samples collected from Elampillai, Tamil Nadu. Shellina and Nishi (2015) identified the microorganisms till the

species level from the textile industries in the western area of Rajasthan specifically Jodhpur, Pali and Balotra. The areas are major hub of textile dyeing and printing. Differences in the microbial growth were observed in soil of the various regions of study. It depends on the availability of nutrient matter, lowered oxygen levels and other organic and inorganic matter present in the dyes. And it also depend on the type of dyes present in the soil and effluent water in that particular area.

Kulandaivel *et. al.* (2014) described the physical, chemical (odor, temperature, pH, TS, TSS, TDS and BOD) and biological (microbial count) properties of six different effluent samples (sewage, textile, coir, whey, leather and metal) of Madurai and found that the different types of microorganisms were present in the waste water (before and after treatment) namely bacteria and fungi. *Bacillus* species was dominated in the effluent stream, which was due to the favorable conditions of oxidizable organic matter.

Prabha *et. al.* (2016) evaluated the impact of application of textile effluent on microbial populations which were high in the untreated effluent as compared to the treated one by conventional treatment systems. Similar trends were observed for Membrane bioreactor (MBR) treatment system as well. *Pseudomonas sp.* (bacterial species) and *Aspergillus fumigates* (fungal species) were found exclusively at the industrial site.

2.3: Seed germination, Growth and yield parameters of crops

Seed germination and seedling growth are important events in the life cycle of higher plants. Number of factors influences the seed germination and seedling growth. Water is one of the most essential factors for seed germination. Any change in quality and quantity of water affects this early event of the plants.

It is well known that water pollution by industrial effluent causes an adverse effect on crop establishment, so industrial effluent i.e. polluted water is considered as adverse factor for seed germination and seedling growth. The germination of seed is one of the most critical phases in the life cycle of plants as it is subjected to several environmental stresses (Sen1977). Numbers of workers have studied the effects of industrial effluent on seed germination. An attempt has been made here to review the work done on effect of industrial effluent on seed germination and seedling growth.

Review of Literature

Plants are sensitive towards the environmental changes and act as better indicator of environmental changes than many of the conventional methods. Seed germination and Seedling growth are important events in the life cycle of higher plants. Numbers of factors influences the seed germination and Seedling growth. Water is one of the most essential factors for seed germination. Any change in quality and quantity of water affects this early event of the plants. It is well known that irrigation of many crops like *Phaseolus aureus*, *Abelmoscus esculentus* and *Hordeum vulgare* with a higher concentration of Industrial effluents I have a negative effect on seed germination and crop establishment. Higher education with law diluted effluent of concentration has a positive effect on seed germination and plant growth (Mohammed and Khan 1985; bahadur and Sharma 1990).

Mohammad and Khan (1985) depicted adverse effects of textile effluent (75 & 100% concentrations) on the germination of *Phaseolus aureus* and *Abelmoscus esculentus* seeds, while there was no negative effect was found upto 50% concentration of the same effluent. Somashekar *et al.* (1984) reported around 40 to 75% inhibition in Bajra and Jowar and around 90% inhibition in paddy crops growth with response to textile industrial effluents.

Jain and Kumari (1990) also outlined the effect of textile printing industrial waste on seed germination and Seedling growth and total Biomass of *spinnaker* L and found that increase in the concentration of effluent causes decrease in the percentage germination. Best selling growth took place in 2.6 and 5 % concentration of effluent.

Swaminathan and Vaidheeswarn (1991) described biochemical changes in peanut crops, and observed that 50% diluted textile effluent increased the seed germination, total sugars, amino acids, phenol and proline starch, protein and chlorophyll than control (distilled water) of peanut seedlings. These studies showed that effects of an industrial effluent vary from crop to crop.

Ramasubramanian *et. al.* (1993) studied the impact of dye industrial effluent on *Phaseolus mungo* L. and reported a declining trend in pigment content. The same was reported in *Vigna mungo*, by distillery effluent. Similar reduction in pigment level was observed in many plants by various industrial effluent irrigation.

Review of Literature

In this study the increase of protein, carbohydrate might be due to the increase of chlorophyll pigments, because of the optimum level of chemicals like sulphate, chlorides, calcium, potassium, phosphorous and magnesium in textile industrial effluent.

Vijayarengan and Lakshmanachary (1993) worked out on the effect of textile mill wastewater on growth and pigment content of green gram cv. ADT3 seedlings at Annamalainagar (India) and reported that the germination percentage of the seeds decreased with an increase in wastewater concentration. Wastewater at lower concentration i.e. 5% and 10% increased the growth and dry weight of the seedlings. While higher concentrations caused deleterious effect on seedlings. The same result was observed for pigment contents.

Shanmughavel (1993) evaluated that the impact of dyeing and sewage effluents on germination of green gram and maize seeds. The dyeing effluent delayed in the germination process and also reduced both the germination percentage and germination value. Soil analysis also indicated that the pollutants like alkali dyes and oil were notably rich in the polluted soil.

Kumawat *et. al.* (2001) at Ujjain (India) investigated the effect of dye and printing industry effluent on germination and growth of chickpea and wheat. They observed that effluent had no such adverse effects on germination at lower concentrations, but yet affect the growth of the different crop cultivars. They proposed that proper crop cultivar selection should be done before using the effluent for irrigation purpose.

Chinnusamy *et. al.* (2001) observed that root and shoot length, fresh weight of root and shoot, dry weight of root and shoot, germination relative index, vigour index and chlorophyll content were higher in 25% than 50% over control. Anoop Singh *et al.* (2002) also observed more chlorophyll content in wheat leaves at 50% effluent irrigation over control, while 100% effluent irrigation resulted in reduction of the same.

Vijayakumari (2005) conducted a pot culture experiment to assess the impact of various concentrations (25, 50, 75 and 100%) of the textile industry effluent on growth response of *Eleusine coracana* L. and normal tap water was

Review of Literature

used as control and reported good growth of root length, shoot length, leaf length, root volume, fresh and dry weight of shoot on 60 days. The germination percentage was 100 in control and 25 in effluent treated pots.

Rajeshwari *et. al.* (2005) reported effects of effluents from a medium sized dye house on plant growth and soil characteristics. They found that diluted effluent enhanced the plant growth while deleterious effects were noticed at higher levels. Accumulation of various substances was also formed in the soil. In general the effluent was not suitable for irrigation.

Gupta and Bishwas (2005) investigated the effect of effluent of a dye industry at Varanasi (India) on the seed germination, seedling growth and chlorophyll content of *Withania somnifera*. They found that the increasing concentration of the effluent induced gradual reduction in the germination percentage and seedling growth. Physico-chemical characteristic of the dyeing industry effluent were also analyzed.

Kumar *et. al.* (2006) studied the impact of various concentrations (0, 25, 50, 75 and 100 %) of textile mill effluent on seed germination and seedling growth of the *Arachis hypogea*. Undiluted effluents had an inhibitory effect (28.9 %), whereas 25% effluent had a growth promoting effect (4.7 %) which was significantly better than control.

Garg and Kaushik (2008) evaluated the suitability of textile mill waste water at different concentrations on growth of sorghum cultivars. The textile effluent did not show any inhibitory effect on seed germination at lower concentration (6.25%). They also observed that seed germinated at hundred percent concentration of effluent but did not survive for longer period.

Egbeeni *et. al.* (2009) reported that fruit production in okra was significantly reduced on application of higher concentrations of industrial effluent. While, positive effect on grain and straw yield in response to application of 20% and 10% diluted textile wastewater could be either due to reduced toxic effect of pollutants. Saravanamoorthy and Ranjitha Kumari (2007) reported a gradual increase in peanut pod yield with the application of effluent of textile industry up to

Review of Literature

50% concentration. Baskaran *et al.* (2009) studied the effect of various concentrations (10, 25, 50, 75 and 100 %) of sugar mill effluent on *Vigna radiata L.* and reported that the amino acid content increased in 10% effluent concentration and then decreased at higher concentration.

Yousaf *et. al.* (2010) investigate the effect of different concentration of textile industry effluents (10,20,30,40,50 and 60%) on germination and growth response of five varieties of *Glycine Max* and observe maximum improvement in Seedling length with the application of 60% of textile effluent in three varieties (NARC-2, NARC-7, Williams-82) while for other varieties, lower concentrations enhanced the growth.

Albino Wins and Murugan (2010) Albina 2010 collected effluent from Madura Textile Mills, Vickramasingapuram, Tirunelveli district, Tamil Nadu and reported the effect of textile effluent was studied with respect to germination and growth of black Gram vigna mungo L. Hepper. In lower concentration of the textile effluent the germination ratio and growth rate are relatively higher than the control, but gradual decrease in the germination of seeds, seedling growth with increase in effluent concentration was observed the best germination and seedling growth was observed in 25% concentration weather growth promoting effect, and significantly better than control. Beyond 25% effluent, root and shoot length was decreased.

Naaz and Pandey (2010) studied the use of industrial waste water on agricultural lands and chlorophyll a, and b were found to increase with increase in concentration of waste water upto 50%, which declined at the exposure of undiluted waste water.

Khan *et. al.* (2011) tested the impact of textile water on seed germination and some of the physiological parameters in three leguminous crop viz. pea, lentil and gram. Plants exhibited a substantial reduction in total plant growth parameters when growth in high concentrations (50 and 100 %). However, the effect of textile effluent was pormotive weather than inhibitory on these parameters when applied in low concentration (10 and 25 %). Medhi (2011) studied the effect of dye factory effluent at 20% dilution and reported that yield characters are relatively higher than the control, but gradually decrease with the increase in effluent concentration.

Review of Literature

Panaskar and pawar (2011) studies the effect of different concentrations of textile effluent on seed germination and it was found that in 20% effluent concentration at the initial 3 days there was no seed germination of *Sorghum Vulgare*, but on 4th day, once seed got germinated and after that, on 5th 6th and 7th-day 3 seeds seen to be generated .in 60 % effluent concentrations for the initial three days there was no *Sorghum Vulgare* seed germination but on 4th day 1 seed got germinated .in 80 and 100 % effluent concentration applied consideration it was observed that not a single *Sorghum Vulgare* seed got germinated. However at 20 %,60 and 80 % effluent concentration, *Vigna aconitifolia* seed germinated faster than the other concentration and less as compared to the control. Malviya et. al. (2012 reported impact of Dyeing Factory effluent on germination and growth of pea (*Pisum Sativum*) and founded lower concentrations of effluent up to 20% short positive impact on germination parameters such a speed of germination and germination index of *Pisum sativum*.

Malaviya et. al. (2011) studied the impact of dyeing industry effluent on germination and growth of pea (*Pisum sativum*), Jammu and reported that the 100% effluent showed high pH (10.3) and TDS (1088 mg l-1). The germination parameters included percent germination, delay index, speed of germination, peak value and germination period while growth parameters comprised of root and shoot length, root and shoot weight, root-shoot ratio and number of stipules. The study showed the maximum values of positive germination parameters viz. speed of germination (7.85), peak value (3.28), germination index (123.87) and all growth parameters at 20% effluent concentration while the values of negative germination parameters viz. delay index (-0.14) and percent inhibition (-8.34) were found to be minimum at 20% effluent concentration.

Varma and Sharma (2012) investigated the effect of different concentration of textile effluent (0,25,50,75,100 %) on the growth of the wheat plant and found that maximum seed germination percentage was in 25% concentration and decreasing gradually as the concentration increased shoot length, weight and number of seed also decrease with increase in the concentration of effluents.

Jolly et. al. (2012) studied the impact of dyeing industry effluent on soil and crop and found that plant height, leaf area, seed dry weight, root dry weight,

Review of Literature

number of seeds obtained from the wheat plant, protein and carbohydrate content in wheat seeds obtained from the plant irrigated with 2.5 and 5% treated effluent also showed an increasing trend and decreased from 10% and above.

Kathirvel P. (2012) collected raw effluent from Slochana dyeing factory, Pallipalayam, Namakkal District, Tamil Nadu and reported that the effect of factory effluent was studied with respect to germination and growth of Bengal gram *Cicer arietinum* L. In lower concentration the germination percentage and its growth enhance than the control, but gradual decrease in the higher concentration. It was observed that the best germination, seedling growth, number of root nodules, yield and biochemical attributes was observed in 20% concentration with growth promoting effective and significantly better than control beyond 20% effluent, root and shoot length decreased.

Mehta and Bhardwaj (2012) described the effect of industrial effluents on seed germination and seedling growth of *Vigna radiata* and *Cicerarietinum*. Germination percentage and seedling growth of both plants showed considerable reduction in the case of untreated effluents. Root and shoot length of *Vigna* seedling were reduced by 58.66% to 69.06% respectively, while in *Cicer* the reduction was between 53.62% and 67.91% in untreated effluent. Minimum reduction in root and shoot length was observed in treated effluent in both *Vigna* and *Cicer*. Maximum phytotoxicity was observed in untreated effluent of *Vigna* and *Cicer*. Treated effluent showed minimum phytotoxicity.

Aswathi *et. al.* (2013) studied the impact of different quantities of dyeing industry effluent on the growth, biochemical characteristics and yield of *Abelmoschus esculentus* (Bhindi). Germination percentage, root length, shoot length, the total fresh weight, total dry weight and vigour index of Bhindi were reported higher in sample T₁ (200 mg of residue). The chlorophyll a, total chlorophyll, carotenoid contents and total soluble sugar of Bhindi were found higher in sample T₃ (600 mg). The anthocyanin, free amino acids L-proline, leaf nitrate, peroxidase and catalase of Bhindi were observed higher in sample T₆ (1200 mg). The total soluble protein of Bhindi was reported higher in sample T₂ (400mg). The nitrate reductase content of Bhindi was found higher in sample T₁. The length,

Review of Literature

weight and numbers of fruits of Bhindi were reported higher in sample T₃ with 600 mg of dyeing industry effluent residue. From the results it had been inferred that the growth parameters and yield performance were higher in T₁ (200 mg) and T₃ (600 mg) respectively.

Ravi *et. al.* (2014) focused on find the effluent through germination studies and identification of by chemical concentration in preated black gram crop. Physicochemical characters of textile dyeing industry effluents were studied. Low concentration of the Dye industries waste at 10 % concentration proven that it is a growth promoting substances were present in the effluent but at higher concentration , textile Dye industry effluent is toxic to crop plants and reduces the root length , shoot length, total leaf area , dry weight and yield of black gram.

Divyapriya *et. al.* (2014) worked on biochemical effect of industrial effluence on germinating seed of *Cicerarientum* and concluded that *Cicerarientum* were treated with industrial effluent for 3 days in 10%, 20%, 30%, 40%, 50%, 100% pure tap water as control and treated effluent to compare the effect of industrial effluent on seeds. Germination percentage was high in the control and it decreases beyond 30% dilution. At 100% effluent treatment the seed germination was completely inhibited. The amount of carbohydrate, protein and total free amino acids were comparable with control, their amounts were increased in the 30% effluent treated seeds. Most of the enzymes were stimulated and their activity was found to be enhanced in the 30% effluent treated seeds.

Parameswari (2014) also reported the effect of textile and dye effluent on germination and growth parameters of greengram, blackgram and redgram. Diluted textile effluent with water in 1:3 ratios did not have any adverse effect on growth and vigour index of field crops. Growth parameters like germination percent, root length, shoot length and dry matter production increased when the concentration of the effluent decreased.

Thakur and Singh (2014) studied on effect of Cd on nodulation and leghaemoglobin in soybean and chickpea and found that number of nodules decrease 30%, 35% and 74% (pod filling stage) at 4 μM , 20 μM and 40 μM Cd treatment levels respectively in soybean plant. Thakur and Singh (2014) also

Review of Literature

reported that Lb content of nodules decrease 15%, 39% and 54% (pod filling stage) at 4 μM , 20 μM and 40 μM Cd treatment levels respectively in chickpea plant.

Rathor *et. al.* (2015) investigated the effect of textile effluent with respect to germination and growth of chickpea (*Cicerarietinum* L.) and concluded that the best germination of seeds, seedling growth was observed in 25% concentration with growth promoting effect and significantly better than control. Beyond 25% effluent concentration, root length and shoot length were decrease.

Ramya *et. al.* (2017) studied on the effect of effluent with respect to germination, morphology and biochemical characters of *Arachis hypogaea* L. variety K6 (groundnut). Dilution effluents (25%, 50%, 75% and 100%) and treated effluent were irrigated on *Arachis hypogaea* L. Seed germination and seedling growth were gradually decreased with increase in effluent concentrations but the best seedling germination and growth was observed in treated effluent compared to other doses. All concentrations of textile effluent were injurious and reduction on plant growth and its morphological parameters of hypocotyls length and epicotyls length. Number of root branches was observed in all effluent concentrations and mostly root affected by 100% textile effluent compared control. Biochemical characters of amino acids and protein were also reduction by increase in effluent concentrations. Morphological and biochemical characters were better growth in treated effluent and followed by 25% effluent than all other concentrations of effluent.

Rana and Kumar (2017) investigation conducted in order to study the phytotoxic effects of textile wastewater on germination and early growth of *Triticum aestivum*. Sixteen samples were collected from Bagru (Hub of small scale textile industries) Rajasthan and were analyzed for various physic-chemical parameters including pH, TDS, EC, Chloride, hardness etc. the wastewater were rich in hardness, chloride etc with pH ranges from 2.0 to 8.12. Parameters studied in case of *o. sativa* includes germination percentage, percentage phytotoxicity, Tolerance index, relative length of germinated seedlings, fresh weight and dry weight of seedlings. Germination percentage and seedling growth shows considerable reduction as compared to control with relative germination percentage

reducing to 36.66% as compared to control with 100% germination percentage. Similarly the wastewater showed inhibitory effect on seedling growth with minimum length of root up to 1.03 ± 0.1 cm and shoot up to 0.74 ± 0.05 cm in contrast with control with 6.48 ± 0.01 cm root length and 5.65 ± 0.04 cm shoot length. Effluent with lower concentration showed seedling growth higher than the control.

2.4: Effect of dyeing effluent irrigation on *Rhizobium* species

Krichner and Buchanan, (1926) described that *Rhizobia* are well known for their capacity to establish a symbiosis with legumes. Legumes are unique plants which have the ability to work with certain bacteria i.e. *Rhizobia* to gather available nitrogen from the soil atmosphere and convert it to usable ammonia nitrogen and make it available to the plant. They inhabit root nodules, where they reduce atmospheric nitrogen and make it available to the plant. Biological nitrogen fixation is a component of sustainable agriculture and *Rhizobial* inoculants have been applied frequently as bio-fertilizers. Each major legume group is nodulated by different species of *Rhizobium*. Soybeans are nodulated by *Rhizobium japonicum*.

Sprent, (2001) studied that among plant-microb interactions, legume-*rhizobium* interactions are unique because they supply 80-90% of total nitrogen requirements of legumes. It involves a complex interaction among host, microbial symbiont and environment. Among nitrogen-fixing systems, legume-*rhizobium* symbiosis is one of the most promising and the bacterial species of *Rhizobium* complex are very important.

Pribac and Ardelean (2008) reported that the most convenient method of obtaining *rhizobia* from nature is by isolation from root nodules. Contrary to popular belief, many of the bacteroids in nodules are viable, is impractical to isolate *rhizobia* directly from the soil because of their fastidious growth requirements and the presence of numerous less fastidious fast growing soil microorganisms.

Lindemann (2008) stated that Nodules, on the other hand, generally contain only *rhizobia*. Some nodules, however, may contain more than one strain of *rhizobium* or may even contain other bacteria. Nodules used for isolation should be in good physical condition to reduce the chance of bacteria other than *rhizobia*

Review of Literature

being present. The portion of the nodule containing *rhizobia* can be located by noting the area with the reddish pigment leg hemoglobin. If the nodule is not potentially capable of fixing N₂, the bacteroid area may not be red but will likely differ in color from the remainder of the nodule. A nodule contains *rhizobia* within it but has many other microorganisms on its surface. These surface micro organisms must be prevented from contaminating the *rhizobia* portion of the nodule by surface sterilizing the nodule.

Prasad and Rao (2010) dye decolorizing isolates, *Bacillus* sp., *Klebsiella* sp., *Salmonella* sp., and *Pseudomonas* sp. were isolated from the textile effluent samples collected from Elampillai, Tamil Nadu.

Gachande and Khansole (2011) worked on Rhizobium isolation and reported that Rhizobium japonicum syn. And Bradyrhizobium japonicum were isolated from root nodules of Soybean (*Glycine max L.*) on yeast extract manitol agar (YEMA) medium and its morphological, culture and biochemical characteristics were studied. It was observed that the colonies were circular, light pink, convex, entire and opac. The bacterium was rod shaped, aerobic, non-spore forming and motile. It showed negative biochemical reaction for indole, methyl red, voges-Prosekaur, hydrogen sulphide production, utilization of carbohydrate and gelatin hydrolysis test. While it showed positive biochemical reaction for citrate utilization, catalase and ammonia production test from peptone and urea.

Shahzad *et. al.* (2012) isolated the beneficial nitrogen fixing *Rhizobium* from root nodules of Alfalfa (*Medicago sativa L.*) plant. They collected total fifty (50) nodule samples equally from five different localities of District Quetta Balochistan, Pakistan and were subjected to culture on differential media Bromothymol Blue (BTB) added Yeast Extract Mannitol (YEM). After studies on biochemical and sugar fermentation tests twenty five (25) samples were identified as *Sinorhizobium meliloti*. They concluded that the organism was present in all the alfalfa growing areas and also confirmed the presence of Rhizobia in leguminous fodder.

Pawar *et. al.* (2014) studies on cultural, morphological and biochemical characterization of Rhizobium, isolated from root nodules of soybean and reported

Review of Literature

that colonies of isolates were entire, opaque with regular margin, milky white, translucent, circular in shape, shiny, raised (convex), sticky, musky odour and 2 to 4 mm in diameter. They also reported that the isolates were aerobic, non-spore forming, Gram negative, rod shaped, motile and showed positive reaction for catalase, oxidation test and starch hydrolysis assay.

Deshwal and Chaubey (2014) worked on Isolation and Characterization of *Rhizobium leguminosarum* from Root nodule of *Pisum sativum* L. For this purpose root nodules were collected from young and healthy seedling of *Pisum sativum* L. from farmer's field at different locations of Dehradun district, Uttarakhand state, India. Eighty one *Rhizobium* strains were isolated from the root nodule of *Pisum sativum* and characterized by standard biochemical tests. Average generation time was between 3.0 to 3.6 h which indicated that isolated *rhizobia* were fast grower. All strains were gram-negative and did not absorb red color when cultured in YEMA containing Congo red. Only 11.11% *Rhizobium* strains showed growth in the presence of 8% KNO₃ in the broth. Strains showed urea hydrolysis (9.87%), gelatinase activity (12.34%) and precipitation in calcium glycerophosphate (14.81%). All the strains utilized D-glucose, mannitol, D-fructose, Larabinose as fermentation sugar. Only 16% Rhizobium strains tolerated 2% NaCl. Results confirmed that isolated strains were *Rhizobium leguminosarum*.

Indra Gandhi *et. al.* (2014) worked on textile effluent and dye contaminated soil and isolated various bacterial species *Alcaligenes spp*, *Bacillus subtilis*, *B.pumilus*, *B.cereus*, *B.megaterium*, *B.licheniformis*, *B.alvei*, *B.macerans*, *B.maxima*, *E.aerogens*, *E.coli*, *Klebsiella pneumoniae*, *Micrococcus spp*, *Lactobacillus spp*, *Pseudomonas florescence*, *P.putida*, *Streptococcus spp*, *Staphylococcus spp*, *S. aureus* and *Serratia spp* and identified by using staining and biochemical test. Banerjee *et. al.* (2017) demonstrated that *B. cereus* IB311 has increased the production (20% and 26% in term of average pod number per plant, average seed number per pod, and seed yield per experimental plot) in ground nut (*Arachis hypogaea* var. *Koushal*, G201) and sesame (*Sesamum indicum* var. *Kanak*), respectively.

Review of Literature

Review of work done by the various authors revealed that irrespective of the type of effluent, these could be well utilized for betterment of agricultural crops on proper dilution to evaluate the lethality of the pollutants. This diluted effluent could be used both for invigorating the seed and for further irrigating the crop and can utilize the waste material for betterment of the mankind without causing ill effects to human and animals.

Though a number of studies have been carried out on effect of various effluent on different plants in many part of world, but review of studied literature indicate that few study was done on impact of dyeing and printing effluent. The present investigation has been carried out to evaluate the effect of dyeing and printing effluent on soil microbiology, growth, biochemical, yield parameters and nodulation (*Rhizobial* strain) study, so our study is a good start and would be beneficial for the agriculturist and for the society.

CHAPTER – 3



MATERIALS AND METHODS

MATERIALS AND METHODS

The present investigation has been conducted to study the Physico-chemical characteristics of textile dyeing and printing effluent and effect of its different concentrations on the growth of leguminous plants i. e. *Glycine max* L. and *Medicago sativa* L. Growth parameters, biochemical parameters, productivity parameters, nodulation parameters were studied using various methods and Physico-chemical characteristics of effluent contaminated soil also studied. Besides, Isolation and characterization of bacterial species from contaminated soil and root nodules have also been studied.

3.1: Experimental Plants-

(A) *Glycine max* L. (JS 335) member of family *Fabaceae*, also known as Soya bean. The bean pods and seeds are a source of oil and protein and are good source of vitamin B. Fermented pods are used to make soya sauce and other sauces and soya milk. Inoculation with nitrogen-fixing bacteria is desirable, the strain *Rhizobium japonicum* being specific to soya bean. A bushy herbaceous legume reaching a height of 20-180 cm. Oil from the seeds used as wetting and stabilizing agent in food, cosmetics, and pharmaceutical products. Soy meal is very rich protein feeding stuff for livestock for which there is an increasing demand. Meal and soy bean protein used in manufacture of synthetic fiber, adhesives, textile sizing, waterproofing, fire-fighting foam and many other uses. Soy flour prepared from the whole beans, producing full-fat flour with about 20% oil, which form mechanically-expressed meal gives low-fat flour with 5–6% oil; that prepared from solvent-extracted meal gives defatted flour with about 1% oil. The flour is used in bakery and other food products; and as additives and extenders to cereal flour and meat products, and in health foods. Soybean seeds extract and fresh soymilk fractions have been reported to possess the cosmeceutical and dermatological benefits such as anti-inflammatory, collagen stimulating effect, potent anti-oxidant scavenging peroxyl radicals, skin lightening effect and protection against UV radiation. The oil is also used in paints, linoleum, oilcloths, printing inks, soaps, insecticides, disinfectants and as a bio fuel. The vegetative portions of plant used for silage, hay, pasture or fodder, or may be plowed under as a green manure. The

Materials and Methods

straw can be used to make paper, stiffer than that made from wheat straw. After oil extraction, the soya meal can be used for manufacturing of fiber, adhesives, and textiles.

(B) *Medicago sativa L. (T9)* it belongs to family Fabaceae, also known as alfalfa. It is one of the highest yielding forage legumes. It is grow as a cover crop to reduce erosion. It has medicinal properties and a yellow dye and trypsin inhibitors can be extracted from the seeds. This is valuable in the treatment of jaundice. It is inoculated with an effective strain of *Rhizobium meliloti*. It is erect, much-branched, perennial plant (30-90 cm) with alternate trifoliate leaves. It is deep-rooted, 2-4 m, or more in well-drained soils. Inflorescences are compact racemes up to 40 mm, borne in axils of upper leaves; purple florets 8 mm, typically papilionaceous. It is mainly grown as a fodder crop. Chlorophyll is extracted from the leaves and the flowers are a source of honey. Alfalfa has been recognized for its superior yield and quality in seeded pastures. Alfalfa is the most productive and most widely adapted forage species and is considered the “queen of forages.” Sweet clover act as like clover are also bloat-causing forages. Bloat also occurs occasionally when cattle are grazed on cereal crops; rape; cabbages; leguminous vegetable crops, including peas and beans; and young grass pasture with high protein content. An increasing occurrence of bloat is noted when cattle are grazed on young green cereal crops such as winter wheat, especially if it is heavily fertilized and irrigated. When taken as a supplement, alfalfa is thought to be beneficial in treating diabetes, high cholesterol, arthritis, urinary tract infections, menstrual problems, and an array of other disorders. Alfalfa also contains a number of important vitamins and minerals, including calcium, potassium, iron, phosphorous, vitamin C, and vitamin K. Extracts of plants are useful as antibacterial.

3.2: Collection of dyeing and printing effluent Samples

The site of sample collection was identified at point where the dyeing and printing effluent is discharged from the small textile mills. The effluent samples were collected from the two selected sites of the area in a wide mouth sterilized plastic bottles. The bottles were filled up to the brim and immediately capped. The

PLATE - 6



(A) *Glycine max L.*



(B) *Medicago sativa L.*

Experimental plants

Materials and Methods

bottles were then numbered with the marker. Collected samples were transported to pollution control board, Kota for Physico-chemical analysis. The critical parameters were tested on the same day while other parameters were tested within their time limit. The collected effluent samples were analyzed for physico-chemical parameters for determination of degree of pollution. The standard methods used given in “ Standard methods for the Examination of water and waste water, 17th edition, 1989, prepared and published jointly by American Public Health Association (APHA), American Water Works Association (AWWA) and Water Pollution.

3.3: Collection of soil samples

Soil Samples were collected from the selected sites, 1, 2 and 3. The area from where the soil samples were collected was marked. An approximately 30cm deep “V” shaped trench was dug from which the soil was discarded. From the walls of the trench, soil was scrapped and collected in two sets. The bags were then numbered with the marker and one set was transported to Maharana Pratap University of Agriculture and Technology (MPUAT), Ummmedganj, Kota for analysis, while the other set was stored at 4° C in refrigerator for future reference.

3.4: Physico-chemical analysis of dyeing and printing effluent

In case of the effluent temperature, color and smell of the samples were recorded on the spot from where the samples were collected, while other parameters of effluent samples which were got analyzed were: pH , electrical conductivity, TDS, BOD, COD, Total Hardness, oil and grease etc.

3.4.1: Color

Color is easily visible to the human eyes, even at very low concentration. Due to low fixation of dyes to the fiber, the waste water from the textile dyeing and printing industries are highly colored. The colored effluents discharged from textile dyeing and printing units impart its color to the soil and the synthetic dyes so released gets absorbed in the same. Dyes are not easily biodegradable thereby recalcitrant in the environment. Removal of color (dyes) from the effluent is a major problem in most of textile industries.

3.4.2: Odor

The presence of foul smell in the waste water is also one of the problems in textile dyeing and printing units. The odor imparts negative aesthetic values to the area.

3.4.3: Temperature (APHA, 1998)

Principle:

The temperature is an important factor, as this has an impact not only on chemical and biological reactions taking place in water but also on the aquatic life. It also affects solubility of oxygen, carbon dioxide, bicarbonate and carbonate. Temperature is measured with a glass thermometer, either alcohol/toluene filled or mercury filled, with 0.1°C graduations.

Requirements - Mercury thermometer.

Procedure –

Temperature of water sample was measured in the field. A mercury thermometer was immersed in surface water for 1 minute and temperature reading was recorded.

3.4.4: pH (Electrometric Method) (APHA, 1998)

Principle:

The basic principle of electrometric pH measurement is to determinate the activity of the hydrogen ions concentration by potentiometric measurement using a standard hydrogen electrode or glass electrode and a reference electrode.

Reagents & Standard buffer solutions –

These may be of pH 4.0, 7.0 or 9.2 in pure water.

Procedure -

Water sample was collected in a beaker and pH was measured by dipping the electrodes. Meter reading was recorded.

3.4.5: Electrical Conductivity (Potentiometric Method)

Principle:

Conductivity is a numerical expression of the ability of an aqueous solution to carry the electric current. This ability depends on the presence of ions, their mobility, valence, relative concentrations and on the temperature of measurement. The inorganic acids, bases, and salt solutions are relatively good conductors. Conductivity measurement gives rapid and practical estimate of the variations in the dissolved contents of the water. Conductivity meter is used to measure the conductivity of the sample.

Requirements - Conductivity meter and cell, Beaker.

Standard potassium chloride solution (0.01M) –

0.7456g of dry AR grade KCl is dissolved in freshly prepared double distilled water and made one liter. At 25°C it gives an electrical conductivity of 1.413mmhos/cm (ds/m).The instrument is to be calibrated or checked with this solution.

Procedure –

First of all conductivity meter was calibrated with the help of standard KCl solution and cell content determined. Then EC of the sample solution was measured with the help of Conductivity meter.

3.4.6: Biological Oxygen Demand (BOD) (Wrinkler Method)

Principle:

The Biochemical Oxygen Demand (B.O.D.) of sewage or of polluted water is the amount of oxygen required for the biological decomposition of dissolved organic matter to occur under aerobic condition and at the standardized time and temperature. Usually, the time is taken as 5 days and the temperature 20°C as per the global standard.

Materials and Methods

The B.O.D. test is among the most important method in sanitary analysis to determine the polluting power, or strength of sewage, industrial wastes or polluted water. It serves as a measure of the amount of clean diluting water required for the successful disposal of sewage by dilution. The test has its widest application in measuring waste loading to treatment plants and in evaluating the efficiency of such treatment systems.

The test consists in taking the given sample in suitable concentrations in dilute water in B.O.D. bottles. Two bottles are taken for each concentration and three concentrations are used for each sample. One set of bottles is incubated in a B.O.D. incubator for 5 days at 20°C; the dissolved oxygen (initial) content (D_1) in the other set of bottles will be determined immediately. At the end of 5 days, the dissolved oxygen content (D_2) in the incubated set of bottles is determined.

$$\text{Then, mg/L B.O.D.} = (D_1 - D_2) / P$$

Where,

P = decimal fraction of sample used.

D_1 = dissolved oxygen of diluted sample (mg/L), immediately after preparation.

D_2 = dissolved oxygen of diluted sample (mg/L), at the end of 5 days incubation.

Among the three values of B.O.D. obtained for a sample select that dilution showing the residual dissolved oxygen of at least 1 mg/L and a depletion of at least 2 mg/L. If two or more dilutions are showing the same condition then select the B.O.D. value obtained by that dilution in which the maximum dissolved oxygen depletion is obtained.

Requirements - B.O.D. bottles 300mL capacity, B.O.D. incubator, Burette, Pipette, Air compressor, Measuring cylinder etc.

Reagents - Distilled water, Phosphate buffer solution, Magnesium sulphate solution, Calcium chloride solution, Ferric chloride solution, Acid and alkali solution, Sodium sulphite solution, Reagents required for the determination of D.O.

Procedure –

Placed the desired volume of distilled water in a 5 litre flask (usually about liter of distilled water will be needed for each sample). Add 1mL each of phosphate buffer, magnesium sulphate solution, calcium chloride solution and ferric chloride solution for every liter of distilled water. Seed the sample with 1–2 ml of textile dyeing and printing effluent. Saturate the dilution water in the flask by aerating with a supply of clean compressed air for at least 30 minutes. Highly alkaline or acidic samples should be neutralized to pH 7. Destroy the chlorine residual in the sample by keeping the sample exposed to air for 1 to 2 hours or by adding a few ml of sodium sulphate solution. Take the sample in the required concentrations. Add the required quantity of sample (calculate for 650mL dilution water the required quantity of sample for a particular concentration) into a 1000mL measuring cylinder. Add the dilution water up to the 650mL mark. Mix the contents in the measuring cylinder. Add this solution into two B.O.D. bottles, one for incubation and the other for determination of initial dissolved oxygen in the mixture. Prepare in the same manner for other concentrations and for all the other samples. Lastly fill the dilution water alone into two B.O.D. bottles. Keep one for incubation and the other for determination of initial dissolved oxygen. Place the set of bottles to be incubated in a B.O.D. incubator for 5 days at 20°C. Care should be taken to maintain the water seal over the bottles throughout the period of incubation. Determine the initial dissolved oxygen contents in the other set of bottles and note down the results. Determine the dissolved oxygen content in the incubated bottles at the end of 5 days and note down the results. Calculate the B.O.D. of the given sample.

3.4.7: Chemical Oxygen Demand (BOD) (Open Reflux Method)

Principle:

The organic matter, present in water sample is oxidized by potassium dichromate in the presence of sulfuric acid, silver sulfate and mercury sulfate to produce carbon dioxide (CO_2) and water. The quantity of potassium dichromate used is calculated by the difference in volumes of ferrous ammonium sulfate consumed in blank and sample titrations. The quantity of potassium dichromate

Materials and Methods

used in reaction is equivalent to the oxygen used to oxidize the organic matter of waste water.

Reagents - Distilled water, Potassium Dichromate Solution, Ferrous Ammonium Sulfate, Ferroin Indicator, Sulfuric acid, Mercuric Sulfate, Silver Sulfate

Procedure –

Taken 20 ml sample in a 250-500 ml COD flask. Added 10 ml of potassium dichromate (0.025 N). Added a pinch Ag_2SO_5 (silver sulfate) and HgSO_4 (mercuric sulfate) followed by 30 ml H_2SO_4 . The content then refluxed on hot plate for at least 2 hours. Flask removed, cooled and distilled water added to make the final volume to about 140 ml. 2-3 drops of ferroin indicator mixed thoroughly into final volume and titrated with 0.01 N (confirm) ferrous ammonium sulfate. At the end point blue-green color of contents changed to reddish blue. Blank was runned simultaneously using distilled water in similar manner.

Calculations –

$$\text{COD (mg/l)} = \{(B-T) \times N \times 1000 \times 8\} / \text{Volume of sample (ml.)}$$

Where,

T = Volume of titrant (FAS) used against sample (ml)

B = Volume of titrant (FAS) used against Blank (ml).

N = Normality of titrant (FAS) (0.25).

Equivalent weight of O_2 = 8.

3.4.8: Oil and Grease (Partition-Gravimetric Method)

Principle:

The Oil and grease contents of domestic and certain industrial wastes and the sludge, is of an important consideration in the handling and treatment of these material for ultimate disposal. Knowledge of the quality of the oil and grease

Materials and Methods

present is helpful in proper design and operation of waste water treatment system. The term grease applies to wide variety of organic substance that is extracted from aqueous solution or suspension by hexane. Hydrocarbons, esters, oils, fats, waxes and high molecular weight fatty acids are the major materials dissolved by hexane. All these material have a greasy feel and are associated with the problems in waste water treatment related to grease. In the Partition-Gravimetric method, dissolved or emulsified oil and grease is extracted from water by intimate contact with trichlorotrifluoroethane; petroleum ether (40/60) or hexane.

Reagents - HCl, Trichlorotrifluoroethane (Freon)

Procedure –

Collect about 1 liter of sample and mark sample level in bottle for latter determination of sample volume. Acidity to pH 2 or lower, generally, 5 ml HCl is sufficient. Transfer to a separating funnel. Carefully rinse sample bottle with 30 ml tricholorotrifluoroethane and solvent washing to separating funnel. Preferably shake vigorously for 2 minute. However, if it is suspected for stable emulsion shakes gently 5 to 10 minute. Let layer separate out, drain solvent layer through a funnel containing solvent – moistened filter paper into a clean, evacuated distilling flask. If a clear solvent layer cannot be obtained, add 1 g Na₂SO₄ if necessary. Extract twice more with 30 ml solvent each time but first rinse sample container with solvent. Combine extracts in evacuated distilling flask and mesh filter paper with an additional 10 ml to 20 ml solvent. Distill solvent from distilling flask in a water bath at 70⁰C. Place flask on water bath at 70⁰C for 15 minutes and draw air through it with an applied vacuum for final 1 minute after the solvent has evaporated. If the residue contains visible water, add 2 ml acetone evaporates on a water bath and repeat the addition and evaporation until all visible water has been removed. Cool in desiccators for 30 minute and weight it.

Calculation -

The amount of oil and grease in the sample can be calculated as,

$$\text{Oil and Grease (mg/l)} = (A-B) \times 1000 / \text{volume of the sample}$$

Where,

A = mass of evacuated flask and residue (g)

B = mass of evacuated flask (g)

3.4.9: Total Dissolved Solid (Gravimetric Method)

Principle:

Determination of total solids is made by evaporating and drying of a measured sample in an oven at 105°C for a period of 1 hour. Since water for potable use contains small amount of suspended mater, it is usual to filter a sample of water and determine solids in filtrate by the fore going method. The difference between total solids in unfiltered and filtered samples is taken as measure of the suspended solids is also classified as volatile or organic solids and fixed or inorganic solid.

Requirement - Evaporating dishes, Drying Oven, Standard Filter Paper, Digital Weighing Balance (microgram), Conical Flask, Measuring Cylinder.

Procedure –

Take a clear dry glass beaker (which was kept at 103°C in an oven for 1 hour) of 150ml. capacity and put appropriate identification mark on it. Weight the beaker and note the weight. Take a 100 ml. of sample and filter it through a double layered filter paper and collect the filtrate in a beaker. Place the beaker in an oven maintained at 103°C for 24hours. After 24 hours, cool the beaker and weight. Find out the weight of solids in the beaker by subtracting the weight of the clean beaker determined in step (1). Calculator total dissolved solids (TDS) as follows:

Calculation –

$$\text{Total Dissolved Solids in mg/l} = \frac{(A - B) \times 100}{\text{Volume of sample in ml}}$$

3.4.10: Total Hardness (EDTA Titrimetric Method)

Principle:

The hardness of water is mainly due to the presence of carbonates, bi-carbonates, chlorides and sulfate of calcium and magnesium in dissolved form. These salts cause excessive consumption of soap used for cleaning purpose. Sodium soaps react with multivalent metallic cations to form a precipitate, thereby lose their surfactant properties. Total Hardness is composed of two components, temporary and permanent hardness. The temporary hardness is due to the presence of carbonates and bi-carbonates of calcium and magnesium. It can be easily removed by boiling the water or by adding lime to water. The permanent hardness i.e. non-carbonate hardness is due to the presence of sulfates, chlorides and nitrates of calcium and magnesium. It requires special methods of water softening. Calcium and Magnesium form a complex of a wine red color with Eriochrome Black T at pH of 10.0 ± 0.1 . The EDTA has got a stronger affinity towards Ca^{+2} and Mg^{+2} and therefore by addition of EDTA the former complex is broken down and a new complex of blue color is formed.

Reagents –

EDTA solution (0.01 M) – 3.723g of disodium of EDTA was dissolved in distilled water to prepare one liter of solution and stored in polyethylene bottle.

Buffer solution –

a. 016.9g Ammonium chloride (NH_4Cl) was dissolved in 143ml of concentration ammonium hydroxide (NaOH).

b. 1.179g of disodium EDTA and 0.780 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ were dissolved in 50ml distilled water. Both (a & b) solution were mixed and diluted to 250ml with distilled water.

Eriochrome Black T Indicator - 0.40g of Eriochrome Black T was mixed with 100g NaCl (A.R.) and grinded.

Materials and Methods

Sodium Sulphaide – 5.0g of Na₂S.9H₂O or 3.7g Na₂S.5H₂O was dissolved in 100ml of distilled water. Bottle was tightly closed to prevent oxidation.

Procedure –

Take 100 ml of sample of water in a conical flask. Add 1 ml of Ammonia buffer and 1 ml of inhibitor solution to it. Add 3 drops of Erio chrome black T indicator, Wine red color will develop. Titrate with standard E.D.T.A (0.01 M) solution until the color changes from wine red to blue. Note down the volume of EDTA consumed say C₁ ml. Take same amount of deionized distilled water and repeat the same exercise. Let the volume of EDTA consumed is C₂ ml. Net volume of EDTA solution required by water sample is C = C₁-C₂

Calculation -

$$\text{Total Hardness (mg/l) as CaCO}_3 = \frac{\text{EDTA (ml) used ('C')}}{\text{Volume of sample (ml)}} \times 100$$

3.4.11: Calcium Hardness (EDTA Titrimetric Method)

Principle:

Calcium hardness can be determined by increasing the pH value of water to 12, at which magnesium ions get precipitated and EDTA forms stable complex while reacting with calcium ions, resulting in change of color from pink to purple when murexide is used as an indicator.

Reagents –

EDTA solution (0.01 M) – 3.723g of disodium of EDTA was dissolved in distilled water to prepare one liter of solution and stored in polyethylene bottle.

Sodium Hydroxide (1N) – 80g of NaOH was dissolved in water and dilute to 1 litre.

Murexide Indicator – 0.2g of Ammonium purpurate was added to 100g of NaCl (A.R.) and grinded.

Materials and Methods

Procedure –

Take 30 ml of sample water in a conical flask and add 2-3 crystals of carbamate and 5 ml of 16% Sodium hydroxide solution. Add 1 ml NaOH to raise pH to 12.0 and a pinch of murexide indicator. Titrate with EDTA till pink color changes to purple. Note the volume of EDTA used say D1 ml. Take same amount of deionized distilled water and repeat the exercise. Let the volume of EDTA consumed is D2 ml.

Net volume of EDTA solution required by water sample $D = D_1 - D_2$

Calculation

Calcium Hardness (mg/l) as $\text{CaCO}_3 = 'D' \times 1000 / \text{ml of sample}$

3.4.12: Magnesium Hardness (EDTA Titrimetric Method)

Principle:

When EDTA (ethylenediaminetetraacetic acid or its salts) is added to water containing both calcium and magnesium, it combines first with the calcium. Calcium can be determined directly, with EDTA, when the pH is made sufficiently high that magnesium is largely precipitated as the hydroxide and an indicator is used that combines with calcium only.

Reagents

1. EDTA Solution (0.01N)
2. Ammonium Chloride- ammonium hydroxide buffer
3. Eriochrome Black – T Indicator
4. Sodium cyanide solution 2% or Sodium diethyl dithiocarbamate crystals.

Procedure –

Take a 25 ml of sample water. Add 2-3 crystals of carbamate and 5 ml Ammonium Chloride – ammonium hydroxide buffer. Add 3-4 drops of Eriochrome Black – T Indicator. Titrate it with 0.01 N EDTA solution till the color changes from bright blue or green and no change or wide red color remains behind. Concurrent reading was taken.

3.4.13: Heavy metals

10 ml of the effluent was taken in a 100 ml kjeldahl flask and 25 ml of triple acid mixture (Conc. Nitric acid, Perchloric acid and Sulphuric acid in 3:2:1 ratio respectively) was added and heated for 3-4 hours in a heating mantle until the initial vigorous reaction has subsided and more strongly heated for 4 hours until the nitrous fumes were removed. At least three concentrations of each standard metal solution were selected to find out the expected metal concentration of a sample. Then aspirated each standard in turn into flame and recorded the absorbance. A calibration curve was prepared by plotting the absorbance of standards versus their concentrations. Sample was aspirated and determined its absorbance against blank.

3.5: Physico-chemical and microbial analysis of dyeing and printing effluent contaminated soil sample

For the analysis of various parameters of soil samples following methods were used (Gupta, P.K., 2007), Bradley (1999) and APHA (1998).

3.5.1: Soil Analysis

1. First of all, soil samples were dried in shade.
2. Then crush the soil clods lightly and grind with the help of wooden pestle and mortar.
3. After this pass the entire quantity through 2 mm stainless steel sieve.
4. Discarded the plant residues, gravels and other material retained on the sieve.
5. For certain type of analysis (example: Organic carbon) grind the soil further so as to pass it through 0.2-0.5 mm sieve.

3.5.2: Analytical Work (Physico-Chemical)

The soil samples were analyzed within a period of three month after collection of soil samples. The method for physico-chemical analyzed in MPUAT, Ummedganj, laboratory, in Kota. All the samples were analyzed for physico-chemical parameters viz pH, Electrical Conductivity, Organic Carbon, Calcium, Magnesium, Nitrogen, Phosphorus, Potassium, Water holding capacity, Heavy metals (Zn, Cu, Mn, Fe).

3.5.2.1: pH (Electrometric Method)

Principle:

The pH electrode used in the pH measurement is a combined glass electrode. It consists of sensing half-cell and reference half-cell, together form an electrode system. The sensing half cell is a thin pH sensitive semi permeable membrane, separating two solutions, viz., the outer solution, the sample to be analyzed and the internal solution enclosed inside the glass membrane and has a known pH value. An electrical potential is developed inside and another electrical potential is developed outside, the difference in the potential is measured and is given as the pH of the sample.

Requirement - pH meter, Standard flasks, Magnetic Stirrer, Funnel, Beaker, Wash Bottle, Tissue Paper, Forceps, Buffers Solutions of known pH value, Distilled Water

Procedure –

Perform calibration of the pH meter using standard pH solutions. The calibration procedure would depend on the pH range of interest. In a clean dry 100 ml beaker take the soil sample and place it in a magnetic stirrer, insert the teflon coated stirring bar and stir well. Now place the electrode in the beaker containing the soil sample and check for the reading in the pH meter. Wait until you get a stable reading. Take the electrode from the water sample, wash it with distilled water and then wipe gently with soft tissue.

3.5.2.2: Electrical Conductivity (Potentiometric Method)

Principle:

Conductivity is a numerical expression of the ability of an aqueous solution to carry the electric current. This ability depends on the presence of ions, their mobility, valence, relative concentrations and on the temperature of measurement. The inorganic acids, bases, and salt solutions are relatively good conductors. Conductivity measurement gives rapid and practical estimate of the variations in the dissolved contents of the water. Conductivity meter is used to measure the conductivity of the sample.

Materials and Methods

Reagent - 0.01 N Potassium chloride solution

Procedure -

First of all conductivity meter was calibrated with the help of standard KCl solution and cell content determined. Then EC of the sample solution was measured with the help of Conductivity meter.

3.5.2.3: Organic Matter (Walkley And Black Rapid Titration

Method, 1934)

Principle:

The organic matter in the soil gets oxidized by potassium and concentrated sulfuric acid utilizing the heat of sulfuric acid. The excess potassium dichromate, not reduced by the organic matter of the soil is determined by black titration with standard ferrous ammonium sulfate.

Reagent -

1. Standard 1 N Potassium dichromate
2. 0.5 N Ferrous ammonium sulfate
3. Diphenylamine indicator
4. Concentrated sulfuric acid
5. Ortho phosphoric acid

Procedure -

1gm of dried soil was taken in 500 ml conical flask. 10 ml of 1 N Potassium dichromate was added and swirled a little. 20 ml of concentrated sulfuric acid was added and swirled a little. The flask was allowed to stand for 30 minutes and then 200 ml of distilled water was added. 10 ml of Orthophosphoric acid was added. Then 1 ml of diphenylamine indicator was added. The content was titrated against 0.5 N ferrous ammonium sulphate solution. Simultaneously a blank was run without soil.

Materials and Methods

Calculation –

$$\text{Organic carbon \%} = \frac{(B - C) \times 0.003 \times 100}{\text{Weight of soil (gm)}}$$

$$\text{Organic matter \%} = \text{Organic carbon \%} \times 1.724$$

Where,

N = Normality of FAS

B = Volume of 0.5 N FAS required to neutralize 10 ml of 1 N Potassium dichromate i.e. blank reading.

C = Volume of 0.5 N FAS needed for titration of soil sample.

0.003 = 1 ml of N Potassium dichromate will be equal to 0.003 gm carbon.

1.724 (Van Bemmlen Factor)

3.5.2.4: Water Holding Capacity (Soak and Drain Method)

Principle:

Water Holding Capacity of soil usually refers to amount of maximum water which can be held by the saturated soil. It is generally measured as the amount of water taken up by unit weight of dry soil when immersed in water under standardized conditions.

Requirement - Hot air oven, Perforated circular soil boxes “Keen – Box”, Filter paper, Petri dish, Balance.

Procedure –

A filter paper was kept in the Keen box of an appropriate dimension to cover the whole perforated bottom of the box. Then the weight of Keen – box plus filter paper was taken as (W_1). The dried and crushed soil was transferred to the Keen – box and weight was taken (W_2). The box was placed in a Petri dish of 10 ml diameter containing water and kept for overnight so that water was entered in the box and saturates the soil. Next day, the box was taken. Wiped and weight was recorded as (W_3).

Materials and Methods

Calculation –

$$\text{WHC \%} = \frac{(W_3 - W_2)}{(W_2 - W_1)} \times 100$$

Where,

W_1 = Weight of box + filter paper

W_2 = Weight of box + filter paper + soil

W_3 = Weight of soil

3.5.2.5: Calcium (Titrimetric Method)

Principle:

Soluble Ca is obtained by extracting the soil by water and measurement of their concentrations in the extract by titration with EDTA (Richards, 1954), resulting color changes from orange to reddish violet (purple).

Reagents –

1. Standard 0.01 N Calcium solution
2. EDTA solution (0.01 N)
3. Murexide Indicator
4. Sodium diethyl dithiocarbamate crystals
5. Sodium hydroxide (4N)

Procedure –

Take a 5-10 ml aliquot of soil water extract and add 2-3 crystals of carbamate and 5 ml of 16% Sodium hydroxide solution. Add 40-50 mg of indicator powder. Titrate it with 0.01 N EDTA solutions till the color gradually changes from orange to reddish violet (purple). Concurrent readings were taken.

3.5.2.6: Magnesium (Titrimetric Method)

Principle:

Soluble Mg is obtained by extracting the soil by water and measurement of their concentrations in the extract by titration with EDTA (Richards, 1954), resulting color changes from bright blue or green and no change or wine red color.

Reagents –

1. EDTA solution (0.01 N)
2. Ammonium chloride – ammonium hydroxide buffer
3. Eriochrome Black – T Indicator
4. Sodium cyanide solution 2% or Sodium diethyl dithiocarbamate crystals

Procedure –

Take a 25 ml aliquot of soil water extract. Add 2-3 crystals of carbamate and 5 ml Ammonium chloride – ammonium hydroxide buffer. Add 3-4 drops of Eriochrome Black – T Indicator. Titrate it with 0.01 N EDTA solutions till the color changes from bright blue or green and no change or wine red color remains behind. Concurrent readings were taken.

3.5.2.7: Heavy Metals

Principle:

In flame atomic absorption spectrometry (AAS), a sample is aspirated into a flame and atomized. A light beam is directed through the flame, into a monochromator, and onto a detector that measures the amount of light absorbed by the atomized element in the flame. The amount of energy at the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample over a limited concentration range.

Requirement -Atomic Absorption Spectrophotometer(GBC, Avanta 932)

Materials and Methods

Reagent - Concentrated nitric acid (HNO₃), Perchloric acid (HClO₄)

Procedure –

1000 ml of acid-treated water sample was taken in a conical flask. 5 ml of conc. HNO₃ was added and covered with a ribbed watch glass. Then the sample was evaporated to 15 to 20 ml on a hot plate. After that, 10 ml of each of HNO₃ and HClO₄ were added, cooled beaker between additions. Then the sample was evaporated again on a hot plate until dense white fumes of HClO₄ just appeared. Sample was cooled, diluted to about 50 ml with metal-free distilled water and then filtered through Whatman No. 42 filter paper. Filtrated was transferred to a 100 ml of volumetric flask and diluted to mark with metal-free distilled water. Reagent blank was prepared following same procedure using metal free water. The clear solution obtained after digestion was analyzed for manganese (Mn), zinc (Zn), iron (Fe) and copper (Cu) by atomic absorption spectrophotometer (GBC, Avanta 932). The metal content in irrigation water is expressed in terms of mg/l.

Calculation –

Metal concentration in sample, mg/l = (S – B) X 100/1000

Where,

S = AAS reading (concentration) of sample, mg/l,

B = AAS reading (concentration) of reagent blank, mg/l,

100 = volume of digested sample, ml,

1000 = volume of sample used for digestion, ml.

3.5.2.8: Determination of Total Nitrogen by Autoanalyzer

Digestion-

The sample is digested in H₂SO₄ to convert organic N to NH₄⁺. N Digestion block digester with tractor auto temperature controller was used in digestion,

Materials and Methods

Transfer 1to2g mineral soil low in N(60mesh) into a digestion tube and add 10ml concentration H₂SO₄ and mix by swirling, heat at 200°C in digestion block then add one Kjeltab, again heat for 15-20min until Kjeltab dissolves (300°C), Then raise temperature to 375°C and heat until sample turn turquoise(45min), then remove the digestion tubes from block allow to cool for 5min, add about 50ml water and mix well until sample is in solution.

Principle:

In the Kjelteb Auto Analyzer method, NH₄.N (liberated by distillation of the digest with strong alkali) is absorbed in unstandardized H₃BO₃ by titration against standard strong acid (HCl).

Requirement - Kjelteb Auto Analyzer.

Reagent - 40% NaOH solution,

Receiving solution – Dissolve 100g H₃BO₃ in 10 litter water. Add 100ml bromocresol green solution. Add 70ml methyl red solution, then 5ml of 4%NaOH.

Standard acid solution - (0.01M HCl)

Procedure –

Bring the digest up to about 100ml, follow instruments for the Kjelteb Auto analyzer, set the alkali pump to deliver 30ml of 40%NaOH, and then titrate with 0.01M NaOH. Calculate the readings.

3.5.2.9: Determination of Available phosphorus

Principle:

Phosphorus in soil ranges from 0.01 to 0.3 per cent and occurs in several forms and combinations. Extraction method (Olsen *et. al.*, 1954) was adopted to determine available phosphorus. Phosphorus, among the major plant nutrients, plays a key role in the development of the plant, in influencing the maturity of the

Materials and Methods

crops and also in quality and quantity of the crop. In soil phosphorus exists in the form of various types of orthophosphates. A very small fraction of these is available to plants at a given time. Available phosphorus content of soil mainly of Ca, Al and Fe –P. In the neutral or alkaline soil particularly, Ca-P is the dominant fraction.

After extraction from the soil, phosphate in the extract is measured by the reaction of phosphate with ammonium molybdate in an acid medium to form molybdophosphoric acid. The molybdophosphoric acid is then reducing to a blue colored complex through reaction with ascorbic acid. Absorbance readings are taken at a 730nm wave length using a spectrophotometer. A standard curve constructed from absorbance readings of standards is used to deduce phosphate concentration of sample.

Requirements - Spectrophotometer, shaker

Reagents - Sodium bicarbonate (NaHCO_3) 0.5M extracting solution: Dissolve 42g of NaHCO_3 in 1000ml of demineralized or distilled water. Mixed thoroughly adjust the pH of the solution to 8.5 with 1M NaOH solution. Darco-G-60 or equivalent grade phosphorus free charcoal, Ammonium molybdate solution, Ascorbic acid solution, Antimony potassium tartrate solution, Sulphuric acid 2.5M, 40% SnCl₂ solution (stannous chloride), 100mg phosphorus solution and 2 mgL⁻¹ phosphorus working solution.

Procedure –

2.5 gm of soil sample, a pinch of Darco-G-60 and 50ml of Olsen reagent were mixed in a 100 ml conical flask and mixed thoroughly on a mechanical shaker. After filtering 5 ml of ammonium molybdate solution containing 400 ml of 10N HCl per liter was gradually added. CO_2 evolved was driven out by slowly shaking. When frothing completely ceased, distilled water was added, washing done the sides, to bring the volume to about 22 ml thereafter 1ml of freshly diluted SnCl₂ solution was added, shaken a little and volume was made 25 ml then intensity of blue color was read at 600ml (red filter). A blank without soil was run under identical manner.

Materials and Methods

Preparation of standard curve for phosphorus- In a series of 25 ml volumetric flask 0, 0.5, 1.0, 1.5, 2.0 and 2.5 ml of 2mg L⁻¹ phosphorus solution was pipette out and 5 ml of Olsen reagent was added. Further gradually 5 ml of ammonium molybdate solution was added and proceed in same the same way described earlier to develop blue color. Intensity of blue color was measured and a standard curve was drawn by plotting concentration of phosphorus against readings.

Calculation-

$$\text{Available phosphorus (Kg/ha-1)} = \frac{Q \times V \times 2.24 \times 10^{-6}}{A \times S \times 10^{-6}} = \frac{Q \times V \times 2.24}{A \times S}$$

Where, Q = quantity of P in (μg) read on X- axis against a sample a sample reading.

V = volume (ml) of Olsen reagent

A = volume (ml) of aliquot used for color development

S = Weight (gm) of soil sample taken

Thus, Available P (Kg ha-1) = Qx8.96

3.5.2.9: Determination of Available Potassium

The Ammonium acetate method to determine available potassium is based on the determination of these two easily available fractions.

Requirements - Flame photometer, mechanical shaker and pH meter.

Reagent Used - 1N ammonium acetate and standard K solution (1000 mg L⁻¹ K solution)

Procedure-

1gm of soil sample was weighted in a 100 ml conical flask, 25 ml of the neutral 1N ammonium acetate solution was added shaken for five minutes and the solution was filtered through What man No 1 filter paper. Concentration of K in the filtrate was measured using flame photometer.

Materials and Methods

Preparation of standard curve for potassium –

Suitable volumes of standard K solution was diluted to get 100 ml of working standard containing 10, 15, 20, 25, 30 and 40 mg KL^{-1} . Reading of the flame photometer was recorded for each of working standards of K after adjusting blank to zero. A standard curve was drawn by plotting the reading against K concentrations.

Calculation-

$$\text{Available K (Kg ha}^{-1}\text{)} = \frac{\text{C} \times 25 \times 10^{-6} \times 2.24}{5 \times 10^{-6}} = \text{C} \times 11.2$$

Where, C stands for the concentration of potassium in the sample obtained on X- axis, against the reading.

3.5.2.10: Sodium

Principle:

Potassium and sodium ions can be determined quantitatively when they are atomized from solution, led to burner and exited to spectral emission in a flame. Since the intensity of the light emitted by each element depends primarily on the concentration of its atoms in the flame at any given instant, a measurement of the light intensity produced by a given element makes possible the quantitative determination of that element.

Requirements- Flame photometer.

Reagent Used –

a. Potassium chloride 1000 ppm:

Dissolve 1.9117 g dried KCl in distilled water and make to 1 L volume.

b. Sodium chloride 1000 ppm:

Dissolve 2.5422 g dried NaCl in distilled water and make to 1 L volume.

Standard curve solutions:

Prepare the following dilution 10, 20, 30,, 100 ppm from the standard 1000 ppm solution in solution of 1 N ammonium acetate pH 7.0.

Procedure:

- (1) Pipet an aliquot of the solution to be analyzed into a 50 ml volumetric flask, complete with 1 N ammonium-acetate solution (pH 7).
- (2) Determine the potassium concentration by use of the flame photometer and the appropriate calibration curve.

Calculation –

- (1) Concentration of Na in sample-extract can be calculated by slope calculation. For every concentration of the standard solution (10-100 ppm) the concentration is divided by the reading of the apparatus. Mean of the resulting values is the slope.

- (2) Content of Na in soil sample:

Content of Na as mg/100g soil (at 105°C)

3.5.2.11: Microbial diversity analysis

3.5.2.11.1: Isolation and Identification of bacteria-

Principle:

This method is based upon the principle that when material containing microorganisms is cultured and each viable microorganism will develop into a colony, hence the number of colonies appearing on the plates represents the number of living organisms present in the sample. The serial dilution agar plate method is one of the commonly used procedures for the isolation and enumeration of bacteria.

Materials and Methods

3.5.2.11.2: Serial dilution and plate count method-

The isolation process of bacterial species was performed by the ‘serial dilution method’. At first, the stock solution was prepared with 0.85 % NaCl concentration and then serial dilution blanks were prepared in test tubes and marked sequentially starting from 10^{-1} to 10^{-5} dilution and autoclave sterilized. 1 gm of soil sample was dissolved in 9 ml solution i.e. 10^{-1} dilution. 1 ml from this was then transferred to 9 ml of the 10^{-2} labeled test tube i.e. 10^{-2} dilution, using a fresh sterile pipette; and this was repeated for each succeeding step till 10^{-5} . Nutrient Agar (NA) media was used for the isolation of bacterial strains. From 10^{-3} , 10^{-4} , and 10^{-5} dilution tubes, 0.1 ml of dilution fluid was then spread on sterilized Petri plates in triplicates using the standard spread plate technique, for bacterial strain isolation.

The Nutrient agar plates were then incubated at 37 °C for 24 h. After successful growth of microorganisms, characteristics of each distinct colony, e.g., shapes, color, transparency, etc. were determined. Gram stain was performed to observe the cellular morphology and gram reaction of the bacteria. The number of bacterial colonies in the contaminated soil samples was counted and the density was expressed as Colony Forming Units (CFU) as given below:

$$\text{CFU/ml in original sample} = \frac{\text{Colonies counted}}{(\text{Dilution factor}) \times (\text{volume plated in ml})}$$

3.5.2.11.3: Biochemical characterization of the isolated bacteria -

Principle:

Gram stain is differential technique which is used to identify and classify bacteria into gram-positive and gram-negative. Four different reagents are used in the fixed bacterial smear in the sequence: crystal violet (primary stain), iodine solution (mordant), alcohol (decolorizing agent) and safranin (counter stain). Gram-positive bacteria retain the primary stain and appear dark blue or violet whereas the Gram-negative bacteria lose primary stain and counter stained by safranin and appear red.

Materials and Methods

Procedure:

A clean sterilized glass slide was taken and bacterial smear was prepared, it allowed to air dry and heat fixed. The smear was covered with crystal violet for 1 minute. The slide was rinsed with distilled water after one minute. Few drops of Gram's iodine was poured on smear and allowed to stand for 30-60 seconds. Wash of the iodine solution with 95% ethanol until no more color flows from the smear. Wash the slide with distilled water and drain. After that the smear was covered with safranin for 30 seconds. The slide was again rinsed with distilled water, air dried and examined under microscope. Observations were recorded as under:

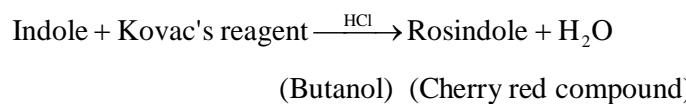
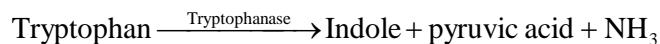
Gram positive - bacteria that were appeared as purple

Gram negative - bacteria that were appered as pink.

3.5.2.11.4: Indole production Test -

Principle:

Some bacteria have tryptophanase enzyme which helps in oxidizing tryptophan (an essential amino acid) and form indole, pyruvic acid and ammonia. Kovac's reagent (dimethylaminobenzaldehyde) is used for detecting the formation of indole during the reaction and confirmed by the presence of cherry-red reagent layer at the top.



Procedure:

To determine the ability of organism to degrade the amino acid tryptophan. The tube containing broths (SIM Agar) were inoculated with isolated bacterial culture. After 24 to 48 hours of inoculation on addition of Kovac's reagent and the results were observed as under.

The color in the top layer of broth tubes was examined.

Materials and Methods

Positive - red color in the top layer (red ring)

Negative - no change

3.5.2.11.5: Methyl Red-Voges Proskauer Test -

Principle:

The two major types of facultative anaerobic enteric bacteria, one that produce large amount of acid and other those produce the neutral end products are used to differentiate by the methyl red (MR) and the voges-proskauer (VP) test. The production of large amount of acids such as formic, acetic, acetic and succinic acid as end product from glucose is detected by the addition of methyl red which act as pH indicator in the medium. The medium remain red and pH remains below 4.4 is the positive test. If the organic acids produced during the glucose fermentation were enzymatically converted to nonacidic end products such as acetoin and ethanol, the methyl red will turn yellow due to the elevation of pH above 6.0 is the negative test.

Procedure:

The tubes containing (MR-VP Broth) were inoculated by isolated bacterial culture. After 24 to 48 hours of incubation on addition of Methly red reagent and addition of Barrit's reagent, results were observed as under:

MR Test Positive - bright red color

 Negative - yellow color

VP Test Positive - pink color, becoming crimson in 30 minutes

 Negative - yellow or colorless

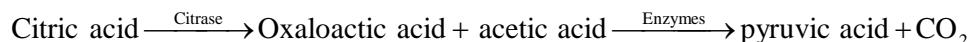
3.5.2.11.6: Citrate utilization Test

Principle:

Some microorganisms produce an enzyme citrase that break down citrate. On the basis of the ability to utilize citrate as a sole carbon source the citrate test is used to differentiate among enteric bacteria. Citrate is break down to oxaloacetic acid and acetic acid which later on by the enzymatically converted to pyruvic acid and carbon dioxide. Sodium citrate in the medium (Simmon's citrate agar) is the only source of carbon and energy. During metabolism of citric acid the CO₂ is

Materials and Methods

formed which combines with sodium and water to form sodium carbonate, which is an alkaline product and changes of the medium from green to blue. This is a positive test. Bromothymol blue is used as an indicator which is green in color at pH 6.8 and below (acidic) and turns blue at pH 7.6 and higher (alkaline).



(Produced during citric acid metabolism)

Procedure:

To find out the ability to ferment citrate as sole carbon source. The tubes containing (Simmons Citrate Agar) were inoculated by isolated bacterial culture. After 24-48 hours of incubation. The results were observed as under:

Positive - blue color and streak of growth

Negative - green color and no growth

3.5.2.11.7: Catalase Test -

Principle:

Microorganisms during aerobic respiration in the presence of oxygen produce hydrogen peroxide (H_2O_2) which is lethal to the cell. The enzyme catalase present in some microorganisms breaks down hydrogen peroxide to water and oxygen and helps them in their survival. Release of free oxygen gas bubbles is a positive catalase test.

Procedure:

Single bacterial colony was transferred on a clear glass slide with the help of loop and few drops of 3% hydrogen peroxide were added immediately. The result was observed as under:

Positive - production of gas bubbles

Negative - no gas bubbles production

3.6: Germination Testing

Seed germination and seedling growth are important events in the life cycle of higher plants. Germination testing is considered as the most important quality test in evaluating the planting value of seeds. The ability of produce normal seedling and plants later on is measured in terms of germination test. Testing of seeds under field conditions is normally unsatisfactory as the results cannot be reproduced with reliability. Laboratory methods than have been conceived wherein the external factors are controlled to give the most uniform rapid and complete germination. Testing conditions in the laboratory have been standardized to enable the test results to be reproduced within limits as nearby as possible as determined by random simple variations.

3.6.1 General Requirements for Germination

Seed require certain conditions for normal germination the most important requirements are substrata, moisture, temperature and light.

Suitable substratum:

The substratum serves as moisture reservoir and provides a surface or medium for which the seeds can germinate and the seedling grow. The commonly used substratum is paper (filter paper, blotter or towel, kraft paper), sand (washed sterilized) and soil (pH 6.0-7.5)

Adequate Moisture:

High concentration of water at cellular level is necessary for the seed to start germination. The moist substrata are sufficient to rehydrate, too much water allow fungal growth and decay of seeds.

Favorable Temperature:

Germination occurs under different ranges of temperatures provided the seed is given adequate, moisture. Seeds of most of the plants germinate in the temperature ranges of 10-35⁰C

Materials and Methods

Light:

Some crops are not required light during germination test. However presence of light is desirable to enable the evaluation of seedling easier and with greater certainty.

Procedure –

Working sample certified seeds were collected from seed certification and testing laboratory, Bajrang nagar, Kota, Rajasthan. 20 seeds were selected as replicates. We have selected two different legume plants that are *Glycine max* L. (JS 335) and *Medicago sativa* L. (T9) for study of germination experiments. All seeds were surface sterilized with 0.1 N mercuric chloride ($HgCl_2$) for 2 minutes and washed with running water to remove contamination of seed coat and prevent any fungal contamination there after the seeds were washed with distilled water. 20 seeds of each plant were taken in Petri plates using blotting paper and a known volume of different concentration of textile dye effluent (20, 40, 60, 80 and 100 %) was poured into different Petri plates and distilled water served as control. Each treatment including control was performed in triplicate and allows them to get germinated. The Petri plates were incubated at 25^0 C in the growth room. Germination was recorded daily at a fixed hourly rate and the emergence of radical was taken as a criterion of germination. For germination percentage paper substrate are used for the following method TP (Top of paper), BP (Between paper). In the present study we use TP method.

TP (Top paper) –

The seeds are germinated on the top of the paper.

Moisture and Aeration –

The substrate must all times contain sufficient moisture and air to meet the requirements for germination. The initial quality of water to be added will depend on the nature and dimension of the substrate and also on the size and species of the seeds to be tested.

Materials and Methods

Categories of seedlings –

Normal seedling is one which shows the capacity for continued development into mature plant when grown in good quality soil and under favorable conditions of water supply, temperature and light. According to International Seed Testing Association (1985) seedling to be classified as Normal seedling, Abnormal seedling, Un-germinate / No-germinate seedlings.

Normal Seedling – Seedling with all their essential structures well developed complete in all proportion and healthy.

Abnormal Seedling – An abnormal seedling is one which does not have the capacity to develop in to a normal plant when grown in the under favorable conditions because one or more the essential structures are irreparably defective. These are damaged seedling (any of the essential structure is missing), deformed or unbalanced seedling (weak and unbalanced development), decayed seedling (essential structures are diseased or decayed due to primary infection).

No-germinated Seed – Seeds which have not germinated by the end of the test period when tested under favorable conditions they may be hard seed, fresh seed, dead seed. In present studies non germinated seeds are of third category i.e. dead seeds. It is conformed as dead seed collapses and a milky paste comes out when passed at the end of the test.

S. No	Crop Botanical Name	Common Name	Substrata	Temp. (°C)	First Count	Final Count	Other Treatment
1.	<i>Medicago sativa</i> (T9)	Rijca	TP	20-30	7 Days	14 Days	Non
2.	<i>Glycine max</i> (JS 335)	Soybean	TP	25-30	7 Days	14 Days	Non

Source: International Seed Testing Association (1985).

3.7: Growth performance:

In the present study the morphological parameters of *Glycine max* L. and *Medicago sativa* L. in control and various treatment levels were studied at 30 days intervals. The various treatment levels (20%, 40%, 60%, 80% and 100%) of textile

Materials and Methods

dyeing and printing effluent solution were prepared and used for Pot culture studies. Twenty seeds were sown in each of triplets in control and various treatment levels. The pots were irrigated with equal volumes of various treatment levels of dyeing and printing effluent. Control set was irrigated with equal volume of tap water. Five plants of both leguminous species were collected from each treatment and they were analyzed for their morphological parameters such as-

- Root length (cm) and Shoot length (cm)
- Fresh weight (g/plant)
- Dry weight (g)
- Vigour index

3.7.1: Length of Shoot and Root –

The morphological parameters like root length and shoot length of *Glycine max* L. and *Medicago sativa* L. were recorded on the 30th days and completion of life cycle after sowing respectively. The root and shoot length were determined using centimeter scale.

3.7.2: Shoot and Root fresh and dry weight –

Ten sample of each treatment were weighted in order to determine the fresh weight and then dried in oven at 80° C for 24 hours to obtain dry weight. Fresh weight and Dry weight were recorded in mg.

3.7.3: Vigour index –

The formula suggested by Abdul-Baki and Anderson (1973) was used to calculate vigour index.

Vigour index = germination percentage X (root length* + shoot length*) (* indicate that length of root and shoot in cm.)

3.7.4: Pigment content –

The estimation of chlorophyll and carotenoid content was done according to Arnon's method (1949).

$$\text{Chlorophyll a (mg/g)} = (12.7 \times \text{OD}_{663} - 2.69 \times \text{OD}_{645} \times V) / a \times 1000 \times W$$

$$\text{Chlorophyll B (mg/g)} = (22.9 \times \text{OD}_{645} - 4.68 \times \text{OD}_{663} \times V) / a \times 1000 \times W$$

$$\text{Total Chlorophyll (mg/g)} = (20.2 \times \text{OD}_{645} + 8.02 \times \text{OD}_{663}) / a \times 1000 \times W$$

$$\text{Carotenoid (mg/g)} = (7.6 \times \text{OD}_{480} - 1.49 \times \text{OD}_{510} \times V) / a \times 1000 \times W$$

Where, OD = optical density, V= volume of extract, a= length of light path in cell (1 cm), W = fresh weight in gm

3.7.5: N, P, K contents –

Determination of Total Nitrogen by Kjelteb Auto Analyzer.

Determination of available Phosphorus by Spectrophotometer.

Determination of available Potassium by Flame Photometer.

(Similar as Soil analysis).

3.8: Biochemical content

(A) Protein content

Protein content was estimated by Folin-Lowry's method (1951).

Requirement - Spectrophotometer (systronic UV-VIS), Alkaline sodium carbonate (2% Na₂CO₃ in 0.1NaOH), Copper sulphate-sodium potassium tartrate,(Alkaline solution prepared fresh), Folin-Ciocalteau reagent, Bovin serum albumin.

Procedure-

Protein reacts with folin ciocalteau reagent to give a coloured complex. This color is produced by the reduction of phosphomolybdate by tyrosin and tryptophan of protein by action of alkaline copper. In this method plant residue

Materials and Methods

(sample+trichloroacitic acid) homogenized and dissolved in .1N NaOH and water, then alkaline copper reagent was added to the dissolved residue and 0.5ml of folin-ciocalteau reagent was added rapidly and mixed immediately, 10 min after optical density at 750nm was measured against blank, the amount of protein in samples was calculated with a standard curve prepared from bovine serum albumin.

(B) Free Amino acid content

Free amino acid content was estimated by Moore and Stein method (1944).

Requirement - 0.1 N HCl, ninhydrin reagent.

Procedure-

1g of plant sample was homogenized in 10 ml of 80% ethanol. The homogenate was centrifuged for 10 minutes at 800 g. One ml of the extract was taken in the test tube and add 1 ml of 0.1 N HCl to neutralize the sample. To this, one ml of ninhydrine reagent was added and heated for 20 minutes in a boiling water bath. Later, 5 ml of the diluents solution was added and heated again in water bath for 10 minutes. The test tubes were cooled and read the absorbance at 570 nm in a UV-spectrophotometer.

(C) Estimation of Free Sugars

Free sugar was estimated by Nelson (1944).

Requirement – arsenomolybdate, reagent ‘C’

Extraction - 500 mg of plant materials were weighed and macerated in a pestle and mortar with 10 ml of 80 per cent ethanol. The homogenate was centrifuged for 10 min at 800 rpm. The supernatant was saved. Then, the ethanol is evaporated in a water bath at 50°C. The net content was made up to 20 ml with distilled water and the extract was used for the estimation of reducing sugar.

Estimation –

One ml of extract was taken in a 25 ml marked test tube. 1 ml of reagent ‘C’ was added. Then, the mixture was heated for 20 min at 100°C in a boiling water bath, cooled and 1 ml of arsenomolybdate reagent was added. The solution was

Materials and Methods

thoroughly mixed and diluted to 25 ml with distilled water. The sample was read in the UV spectrophotometer at 520 nm. The sugar contents were expressed in mg/g fresh weight basis.

3.9: Yield parameters

3.9.1: Total number of Pods per plant –

The number of Pods/fruits (Soybean and Alfalfa) from three pots was summed up and the mean was worked out and expressed as number of pods per plant for each treatment.

3.9.2: Weight of Pod per plant –

The Pods weight (Soybean and Alfalfa) gently washed with water, blotted on a filter paper, weighed and the mean weight of the pods from each treatment was calculated and expressed in grams.

3.9.3: Total number of Seeds per pod –

The number of seeds (Soybean and Alfalfa) from three pots was summed up and the mean was worked out and expressed as number of seeds per pod for each treatment.

3.9.4: Weight of Seeds per pod –

The Pods (Soybean and Alfalfa) and pods (cluster bean) and gently washed with water, blotted on a filter paper, weighed and the mean weight of the pods from each treatment was calculated and expressed in grams.

3.10: Nodulation study

3.10.1(A): Collection of plant Nodules

Plant roots of legumes bearing nodules were collected from dyeing and printing effluent treated soil of 40-60 days of plantation. Roots were uprooted and washed in running tap water to obtain clean root nodules. The portion of the nodule containing *rhizobia* can be located by noting the area with the reddish pigment leghaemoglobin.

Materials and Methods

3.10.2: Total number of Nodules per plant

The number of Nodules (Soybean and Alfalfa) from three pots was summed up and the mean was worked out and expressed as number of nodules per plant for each treatment.

3.10.3: Fresh and Dry weight of Nodules per plant

The uprooted Nodules (Soybean and Alfalfa) washed with water, blotted on a filter paper, weighed and the mean fresh weight of the nodules from each treatment was calculated and expressed in grams. Similarly the uprooted Nodules (Soybean and Alfalfa) washed with water, blotted on a filter paper, get dried and the mean dry weight of the nodules from each treatment was calculated and expressed in grams.

3.10.4: Leghaemoglobin content

Leghaemoglobin Content of Root Nodules estimated by Sadasivam and Manickam, (2007) method.

Requirement- potassium hexacyanoferrate, sodium dithionite

Estimation –

The washed and weighed (200 mg nodules) (both effective and ineffective) were crushed in phosphate buffer (pH 7.4), macerated in a mixer separately and then centrifuged at 10,000 x g for 10-30 min. The supernatant was made up to 4.0 ml with phosphate buffer and 2.0 to 5.0 ml of pyridine reagent was added. Then, it was divided equally between two tubes. To one portion was added a few crystals of sodium dithionite and the optical density was measured at 556 nm after 2-5 min in spectronic 20 using the blank without extract. To the other portion, a few crystals of potassium hexacyanoferrate were added to oxidize the hemochrome and the optical density was measured at 539 nm. The quantity of leghemoglobin was calculated by comparing with the standard graph prepared using pyridine and expressed in mg/g.

Materials and Methods

3.10.2(B): Culture Media-

The medium is referred to as yeast extract mannitol agar (YEMA) and contains 10gm of mannitol, 0.5 gm of potassium monohydrate phosphate (K₂HPO₄), 0.2 gm of magnesium sulfate heptahydrate (MgSO₄.7H₂O), 0.1 gm of sodium chloride (NaCl), 0.01 gm of calcium carbonate (CaCO₃) 0.5 gm of yeast extract powder, 15gm of agar, and 1,000 ml of distilled water. Leave the agar out to prepare yeast extract-mannitol broth (YMB). Adjust the pH to 7.0 with 1N hydrochloric acid (HCl) before autoclaving.

3.10.3(C): Isolation of *Rhizobial* Strains-

Plant roots of legumes bearing nodules were collected from 20% concentration of dyeing and printing effluent treated soil pots and healthy pink nodules were collected carefully washed with sterile water followed by surface treatment with 95% alcohol and again with sterile water. The washed nodules were surface sterilized by immersing in 0.1% HgCl₂ solution for 5min. Then nodules were washed with sterilized water 5times to get the sterilizing agent. The surface sterilized nodules were crushed in sterilized Petri plates this nodule suspension was then serial diluted (10⁻⁵ to 10⁻⁷) streaked on the sterilized YEMA medium plates. The plates were incubated for 3 to 8 days at 28°C. *Rhizobium* was obtained from these nodules. Isolated colonies of *rhizobia* were transferred on YEMA medium slopes and stored in the refrigerator for further studies. *Rhizobia* colonies on YEMA may appear in 2 to 4 days for the fast growing *rhizobia* and from 3 to 9 days for the slow-growing *rhizobia* depending on the physiological state of the *rhizobia* at the time of plating.

3.10.3(D): Study on Microbiological Character-

The identity of the isolates as *rhizobium* was established by characterization tests including Gram staining, growth on YEMA medium on Congo red (2.5 ml/l of 0.1% solution), after confirmation as rhizobium strain, pure cultures maintained on basal media were used in the study. All the 4 samples for 2 plants x 2 (control + 20% dyeing and printing effluent amended soil) were screened for their ability to produce different enzymes involved in biochemical reaction following standard methods (Dubey and Maheswari 2002, Aneja 1996).

Materials and Methods

3.10.3(E): Characterization of Authenticated Isolates-

The morphological and cultural as well as physiological or biochemical characteristics of the authenticated isolates were studied following the procedure given by Aneja (1996).

3.10.3(F): Morphological and Cultural Characteristics-

(F-A) Morphological Characteristics-

The size, shape, motility and Gram stain reaction of *rhizobial* cells were observed under microscope using standard procedure.

• Gram Staining of Bacteria –

Gram staining differentiates bacteria by the chemical and physical properties of their cell walls. Gram-positive cells have a thick layer of peptidoglycan in the cell wall that retains the primary stain, crystal violet. Gram-negative cells have a thinner peptidoglycan layer that allows the crystal violet to wash out. They are stained pink or red by the counterstain, commonly safranin.

Make a thin smear of culture on glass slides dried the smear and heat fix, cover the smear one by one with crystal violet (60 sec), gram iodine (60 sec) 95% C₂H₅OH (20 sec) and safranin (40 sec). Air dried slides after washing with Distilled Water and observed under microscope.

• Motility –

Sulphide Indole Motility (SIM) medium agar may also be used to detect motile organisms. Motility is recognized when culture growth (turbidity) of flagellated organisms can be easily visualized and is not restricted to the line of inoculation. Growth of non motile organisms is confined to the line of inoculation.

(F-B) Colony characteristics-

The configuration, margin, elevation and color of the colonies of the test isolates grown on standard YEMA plates were observed.

Materials and Methods

- **Cultural Characteristics-**

Strains were spread over YEMA agar plates were also streaked on YEMA agar. The inoculated plates were incubated at 28°C for 48 hours and observed for colony shape, size, color and texture. The shape, color, opacity, margin and elevation of the colonies of the test isolates grown on standard YEMA plates were observed.

(F-C) Biochemical Characteristics –

Biochemical methods were performed to differentiate the unknown cultures and for this, various tests were performed.

Biochemical characteristics of the Rhizobium isolates were studied using different tests like Indole, Methyl red and Voges Proskauer test, Citrate utilization, Catalase test as described by Aneja (1996). The biochemical tests were carried out in growth medium at 28°C for 48 hours incubation. All the tests were carried out with 03 replicates.

Identification of *Rhizobium*-

To study the fast or slow growing nature of the isolates they were grown on freshly prepared YEMA plates containing bromo-thymol blue adjusting the pH to 6.8.

(F-C.1) Indole Test –

To determine the ability of organism to degrade the amino acid tryptophan. The tube containing (SIM Agar) were inoculated with isolated Rhizobium culture. After 24 to 48 hours of inoculation on addition of Kovac's reagent, change in colour of media was observed.

Materials and Methods

(F-C.2) Methyl Red –

To determine the ability of microorganisms to oxidize glucose with the production and stabilization of high concentration of acid end products. The tubes containing (MR-VP Broth) were inoculated by isolated Rhizobium culture. After 24 to 48 hours of incubation on addition of Methyl red reagent, change in colour of the medium was observed.

(F-C.3) Voges-Proskauer-

The voges proskauer test determines the capability of organism to produce non acidic or neutral end products. The tubes containing (MR-VP Broth) was inoculated by isolated Rhizobium culture. After 24-48 hours of incubation on addition of Barritt's reagent, change in color of the medium was observed.

(F-C.4) Citrate Utilization-

To find out the ability to ferment citrate as sole carbon source. The tube containing (Simmons Citrate Agar) were inoculated by isolated bacterial culture i.e. *Rhizobium*. After 24-48 hours of incubation, any change in the color of the media was observed.

(F-C.5) Catalase Activity Test –

To determine the ability of some rhizobium to degrade hydrogen peroxide by producing the enzyme catalase. Slide containing 2-3 drops of (Trypticase soya broth) were inoculated by 24-48 hours isolated *Rhizobium* culture. After few seconds on addition of 3% hydrogen peroxide observe the change on slide. Bubbles were appeared on the slide. Different isolates which were 48 hour old were flooded with hydrogen peroxide and observed for liberation of effervescence of oxygen around the bacterial colonies according to Graham and Parker (1964).

Materials and Methods

Table 5: Various Types of Media Composition

Medium	Compositions	Quantity	Medium	Compositions	Quantity
SIM Agar Medium	Peptone	30 g	MR-VP Broth	Peptone	7 g
	Beef extract	3 g		Dextrose	5 g
	Ferrous ammonium sulphate	0.2 g		Potassium phosphate	5 g
	Sodium thiosulphate	0.025 g		Distilled water	1000 ml
	Agar	3 g		pH: 6.9±0.2	
	Distilled water	1000 ml			
	pH: 7.3±0.2				

Medium	Compositions	Quantity
Simons citrate agar medium	Ammonium dihydrogen phosphate	1 g
	Dipotassium phosphate	1 g
	Sodium chloride	5 g
	Sodium citrate	2 g
	Magnesium sulphate	0.2 g
	Bromothymol blue	0.8 g
	Agar	15 g
	Distilled water	1000 ml
	pH: 6.9±0.2	
Medium	Compositions	Quantity
Trypticase soya broth	Trypticase	15 g
	sodium chloride	5 g
	Distilled water	1000 ml
	pH: 7.3±0.2	

CHAPTER – 4



OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

Present research work deals with the physico-chemical study of dye effluent and its impact on soil and nodulation in some leguminous plants. The effluent from dyeing and printing units in Kaithun region, Kota affect the growth, productivity, biochemical characteristics of *Glycine max* L. and *Medicago sativa* L. The observations and results of present study were divided in to five parts. The first part covers the physico-chemical characteristics of dyeing and printing effluent. The second part covers the effect of dyeing and printing effluent on soil characteristics. The third part includes the effect of dyeing and printing effluent on germination and seedling growth. Fourth part deals with the evaluation of various growth and biochemical parameters of both the experimental plants treated with effluent. The fifth part deals with the nodulation and microbial study. The following parameters were taken under consideration.

1. Physico-chemical study of dye effluent and effluent contaminated soil.
2. Effect of dyeing and printing effluent on germination and seedling growth.
 - Shoot length
 - Root length
 - Fresh weight of Seedling
 - Dry weight of Seedling
 - Vigour index
3. Evaluation of various growth, biochemical and yield parameters.
 - Shoot length and Root length
 - Fresh weight of Shoot and Root
 - Dry weight of Shoot and Root
 - Vigour index and N, P, K Content in seedling
 - Yield parameters (Pods/plant, Pods weight/plant, Seed/pod, Seed weights/pod)

Observation and Results

- Pigment content (Chlorophyll a, Chlorophyll b, Total Chlorophyll, Carotenoids)
 - Protein
 - Free Amino acid
 - Free Sugar
4. Nodulation study
 - Nodules/plant
 - Fresh and dry weight of Nodules
 - Leghaemoglobin content
 5. Microbial study of contaminated soil and isolation and identification of *rhizobium* bacteria from control and 20% effluent treated root nodules of experimental plants.

The results of the physico-chemical parameters of dyeing industrial effluents, physico-chemical parameters of effluent contaminated soil, growth, biochemical characteristics of crop plants, yield performance of crop plants and nodulation study are discussed here under. All the physico-chemical parameters of dyeing industry effluent are compared to the disposal standard for industrial effluent proposed by WHO, CPCB and BIS. Dyeing industrial effluent contains high values of physico-chemical parameters such as electrical conductivity, biological oxygen demand, chemical oxygen demand, total dissolved solids, cations and anions.

4.1: Physico-chemical characteristics of dyeing and printing effluent

The physico-chemical characters of the effluent from the kaithun region, Kota are presented in Table 6. The color of the dyeing industry effluent was Dark blue and black in sample A and sample B respectively. The temperature of concentrated effluent of dyeing industry was found within the permissible limit i.e. 26°C and 31°C at site ‘A’ and site ‘B’. The effluent of dyeing industry was slightly alkaline in nature with pH value of 7.7 and 7.9 in sample ‘A’ and Sample ‘B’

Observation and Results

respectively, that ranges within the permissible limit (6.5-8.5) prescribed by WHO and BIS. But the pH value of non contaminated water (Control) was found 7.2 which were more alkaline than that of other two concentrated effluent samples. The chemicals used in dyeing industries, like caustic soda and sodium hydroxide might be responsible for the alkaline nature of the effluent.

Electrical conductivity is an important physical parameter to measure the sodium hazard of water quality. The Electrical Conductivity found 1479 $\mu\text{S}/\text{cm}$ and 2200 $\mu\text{S}/\text{cm}$ in sample ‘A’ and sample ‘B’ respectively that was greater than the permissible limit of BIS (300 $\mu\text{S}/\text{cm}$) this may be due to the continuous discharge of the chemicals and salts used along with dyes in the dyeing processes of the industries.

Chemical Oxygen Demand (COD) value is a measure of O_2 demand of water, against total chemicals. It was observed that the COD values were higher than permissible limit (250 mg/l) for sample ‘A’ and sample ‘B’ i.e. (6864 mg/l) and (3120 mg/l) respectively. The COD value of control sample was also calculated i.e. 64 mg/l.

The Biological Oxygen Demand (BOD) is due to the presence of organic contaminants of dyeing and printing effluents in water bodies. Biological oxygen demand is an overall measurement of the biodegradable organic matter in a wastewater indirectly via microbial oxygen consumption. The low or nil BOD shows good quality water, whereas a high BOD indicates the water is highly contaminated. Biological Oxygen Demand (BOD) value was observed very high than permissible limit (250 mg/l) for sample ‘A’ (1090 mg/l), but slightly higher in sample ‘B’ (488 mg/l).

The Total Dissolved Solids (TDS) of the concentrated effluent samples ‘A’ and ‘B’ were (980 mg/l) and (1440 mg/l) respectively. The value of TDS of sample ‘A’ is lower than permissible limit whereas vale of TDS of sample ‘B’ is higher than permissible limit of BIS i.e. 1000 mg/l. Total solid concentration in dyeing effluent may not be very high; however, total load of this parameter may still be significant, as the use of large amount of water in dyeing and printing industry offsets the effect of low concentrations.

Observation and Results

The Calcium Hardness of sample ‘A’ and sample ‘B’ were (800 mg/l) and (110 mg/l) respectively. The calcium hardness of sample ‘A’ is higher than the permissible limit set by BIS 200 mg/l. Magnesium Hardness is also higher than the permissible limit (20-100 mg/l) in sample ‘A’ and sample ‘B’ i.e. (1450 mg/l) and (120 mg/l) respectively. Calcium hardness and Magnesium hardness of control water sample (160 mg/l and 130 mg/l respectively) were slightly higher than the Calcium and Magnesium hardness of sample ‘B’. Total hardness of the effluent sample ‘A’ was very high (2250 mg/l) than the sample ‘B’ (230 mg/l), control (290 mg/l) and permissible limit of BIS (600 mg/l).

In the present study heavy metal analysis was also done but there is no significant concentration of Cu, Zn, Fe and Ni was found. Copper in the effluent sample ‘A’ and sample ‘B’ was 0.02 mg/l and 0.05 mg/l respectively. The concentration of Zinc in sample A and sample B was 0.31mg/l and 0.39 mg/l respectively. The value of Iron and Nickel concentration was 0.27 mg/l and 0.04 mg/l respectively in sample ‘A’ and 0.21 mg/l and 0.01 mg/l respectively in sample ‘B’. All the values are under the permissible limit set by BIS.

Oil and grease values were also recorded for sample ‘A’ and sample ‘B’ i.e. 43.6 mg/l and 21 mg/l respectively which is quite higher than Central Pollution Control Board limits (10 mg/l). The presence of oil and grease in water bodies leads to the formation of oil layer, which causes significant environmental problem such as reduction of light penetration into water system therefore photosynthesis process for water living organism is hindered. Besides, it prevents transfer of oxygen from atmosphere to water medium as a result reduces the amount of dissolved oxygen (DO) at the bottom of the water bodies and disturbs the process of food chain in the aquatic life CPCB (1990).

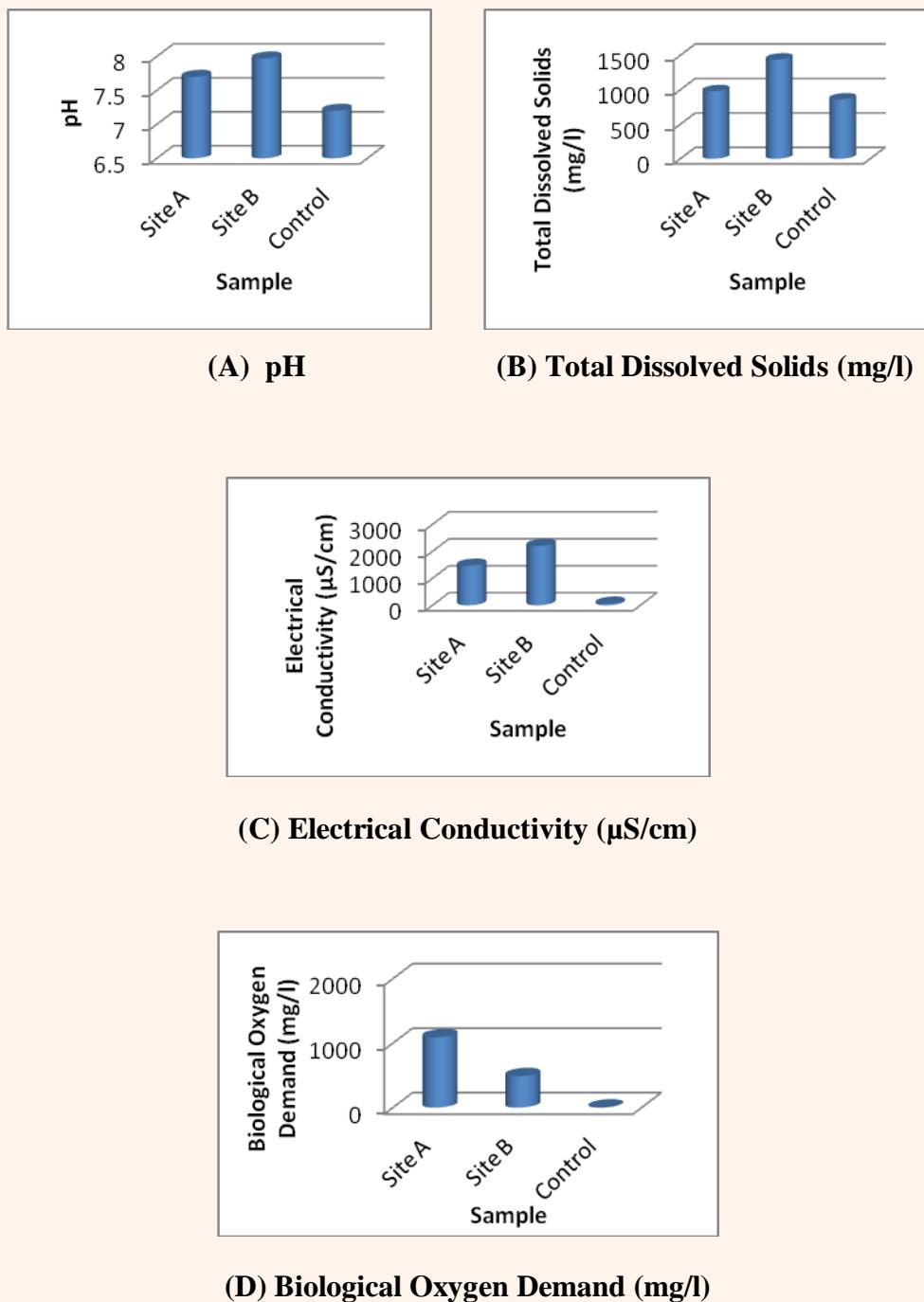
Observation and Results

Table 6: Physico-chemical Characteristics of Dyeing and Printing Effluent of the Study area

Parameters	Units	Site 1	Site 2	Control	CPCB	BIS
Temperature	°C	26	31	15	-	>40
Colour	-	Dark blue	Black	Colorless	-	-
Odour	-	Pungent	Pungent	-	-	-
Ph	-	7.20	7.97	8.2	5.5-9.0	6.5-8.5
Total dissolved solids	mg/l	980	1440	861	-	1000
Electrical conductivity	µS/cm	1470	2200	64	-	3000
Biological oxygen demand	mg/l	1090	488	0.4	100	30
Chemical oxygen demand	mg/l	6864	3120	64	250	250
Calcium Hardness	mg/l	800	110	160	-	75-200
Magnesium Hardness	mg/l	1450	120	130	-	20-100
Total Hardness	mg/l	2250	230	290		600
Oil and grease	mg/l	43.6	21	-	10	10
Copper	Ppm	0.02	0.05	-	-	0.05
Zinc	Ppm	0.31	0.39	-	-	5
Iron	Ppm	0.27	0.21	-	-	0.3
Nickel	Ppm	0.04	0.01	-	5	-

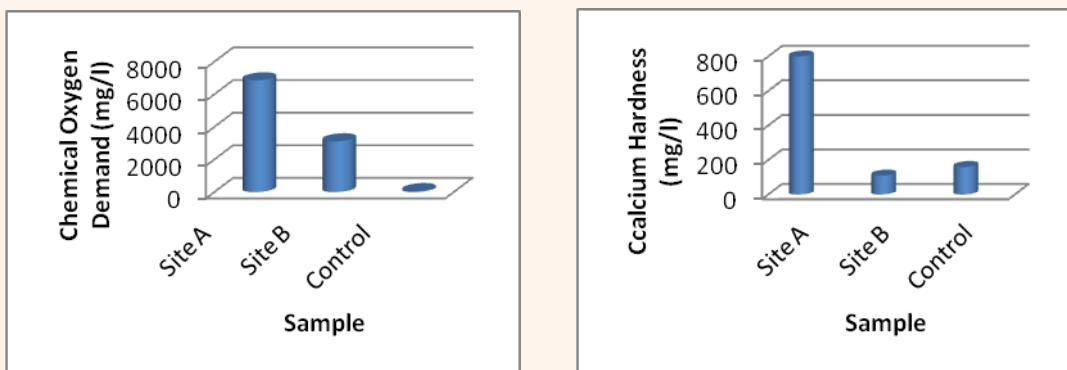
CPCB = Central Pollution Control Board, BIS = Bureau of Indian Standards (10500:1991)

FIGURE - 1

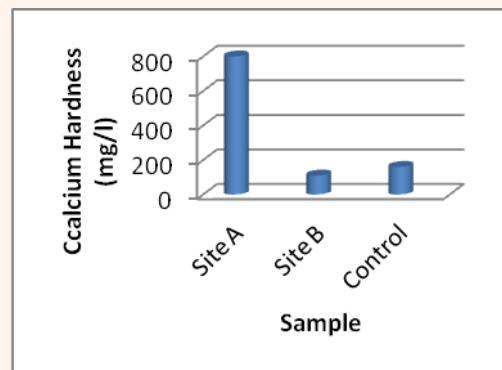


Graph showing Comparative study of Physico-chemical Characteristics of Effluent and Control

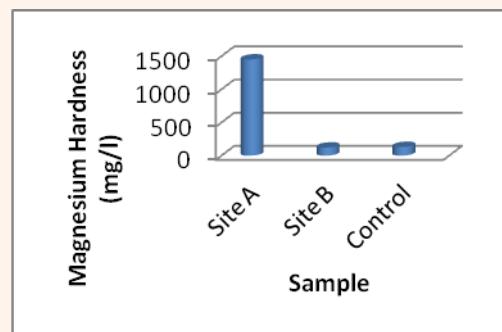
FIGURE - 2



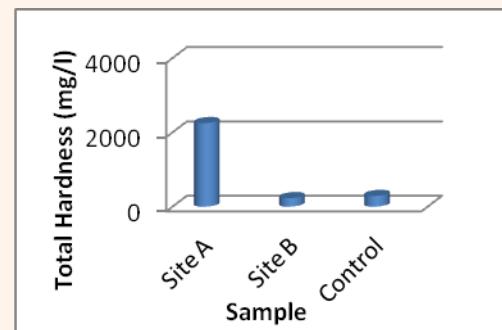
(E) Chemical Oxygen Demand (mg/l)



(F) Calcium Hardness (mg/l)



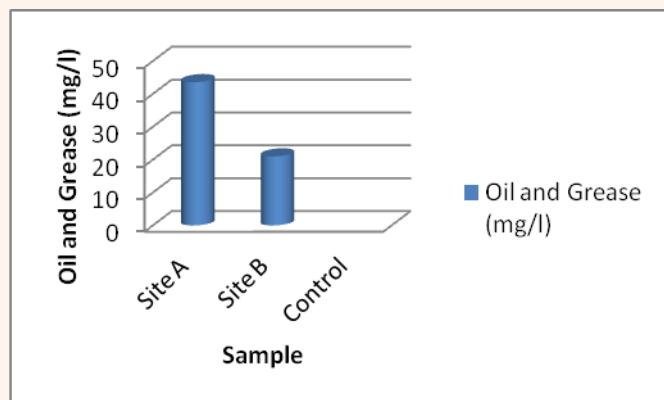
(G) Magnesium Hardness (mg/l)



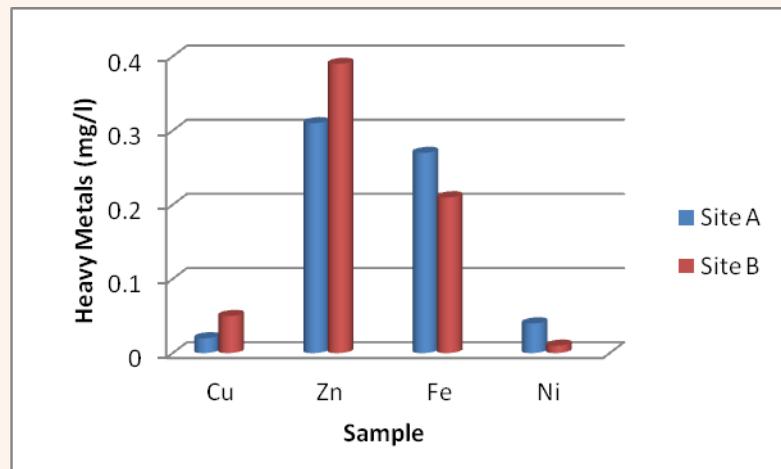
(H) Total Hardness (mg/l)

Graph showing Comparative study of Physico-chemical Characteristics of Effluent and Control

FIGURE - 3



(I) Oil and Grease (mg/l)



(J) Heavy Metals (mg/l)

Graph showing Comparative study of Physico-chemical Characteristics of Effluent and Control

4.2: Effect of the dyeing and printing industrial effluent on soil characteristics

The Physico-chemical parameters of soil contaminated by dyeing and printing effluent are presented in Table 7 and figure 4, 5, 6. The pH of the all samples was ranged from 7.99 to 8.19 that was slightly in the alkaline and within the permissible limit (6.5-8.5) prescribed by WHO and BIS.

In the present study Electrical Conductivity (EC) ranged between 0.21-0.44 ds/m. The EC values for all the samples were well within the normal range but the values of EC of sampling site 2 were higher than the sampling site 1 and 3.

In the present study % organic carbon was recorded which were ranged in between 0.53%– 0.96%. Continous discharge of effluent increases organic carbon content of soil. The nutrients such as sodium (Na), Phosphorus (P) and Potassium (K) were higher in soil of polluted sampling site 1 (effluent dumping sites). The average values of sodium, phosphorus and potassium 6.13 Kg/hac, 54.4 Kg/hac and 573 Kg/hac respectively at site 1. The value of Na, P, and K of sampling site 2 and 3 is less than sampling site 1. The application of effluent or waste water markedly increased the available sodium in contaminated soil.

Water holding capacity shows physical condition of soil, it is the point at which soil gets completely saturated with water. Pollutants and industrial discharge increase the soil water holding capacity (Sheikh and irshaad 1980; Rai *et. al.* 2011). More water holding capacity shows the good physical condition of soil. In present study water holding capacity ranged between 37.91% - 45.86%.

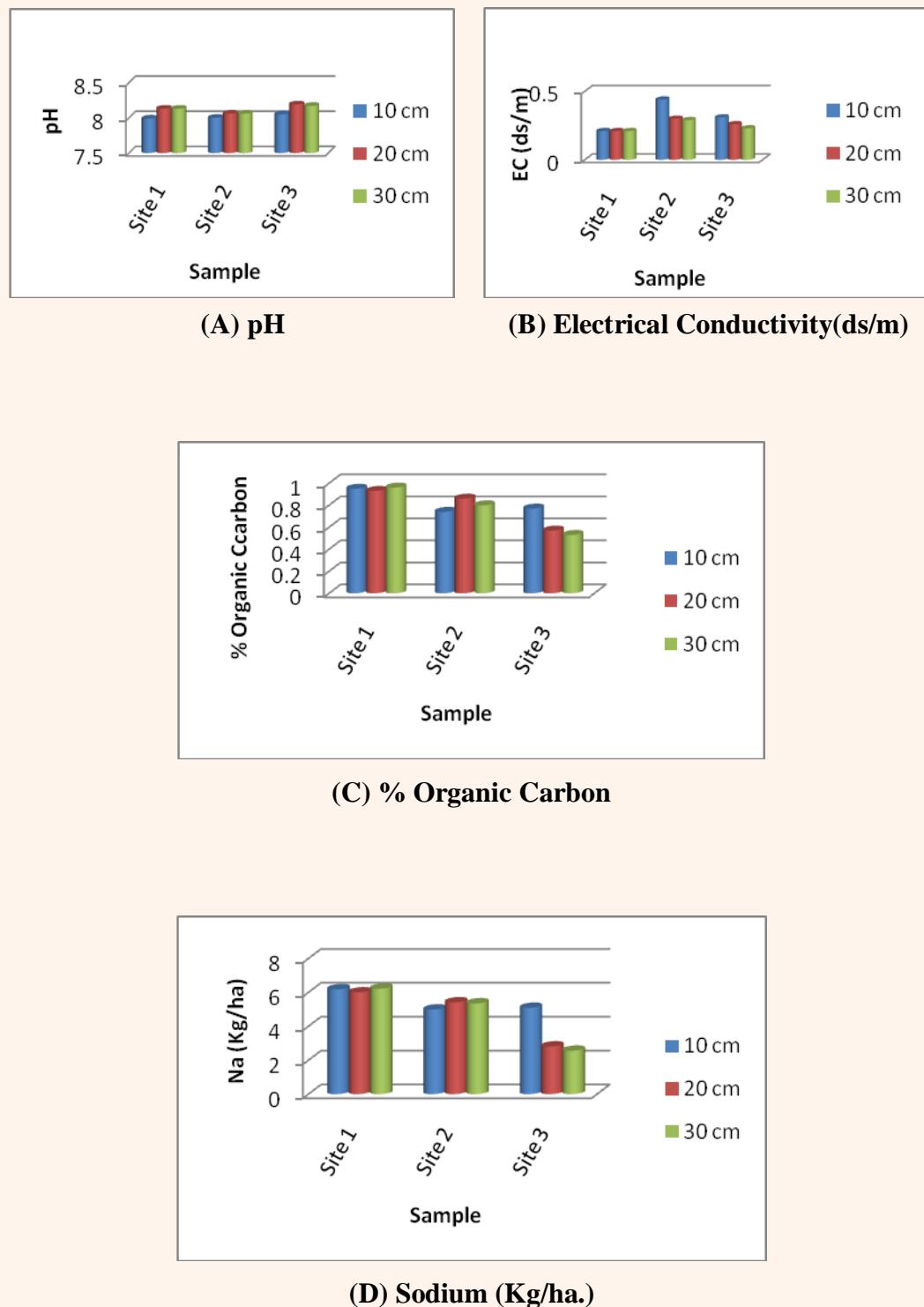
In the present study zinc in the soil samples ranged 0.33-6.88 ppm. Copper content was found in all the soil samples ranged from 0.88-9.33 ppm. Manganese content was found from 4.31-11.50 ppm. Iron in the soil samples ranged from 0.253-9.02 ppm.

Observation and Results

Table 7: Effect of the Dyeing and Printing Effluent on Soil Characteristics

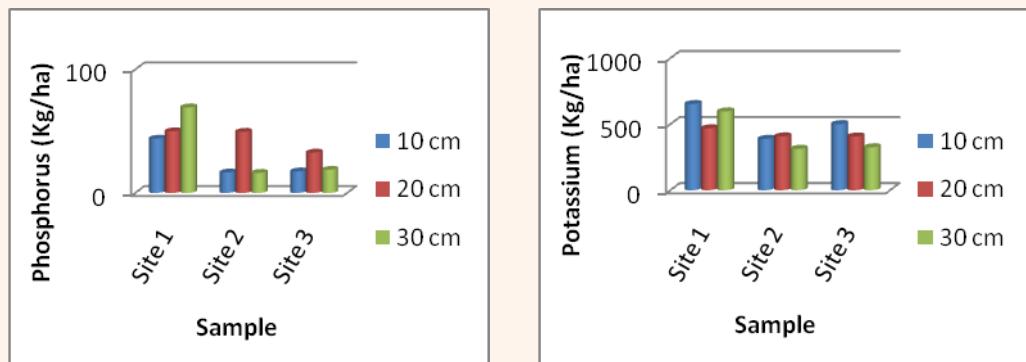
S. No.	Sample	Parameters												
		pH	EC (ds/m)	%OC (%)	Na (Kg/ha)	P Kg/ha	K Kg/ha	Ca (gm/l)	Mg (gm/l)	%WHC (%)	Zn (ppm)	Cu (ppm)	Mn (ppm)	Fe (ppm)
1.	S1													
	10 cm.	7.99	0.21	0.95	6.18	44	653	3.2	3.6	45.27	6.88	4.86	11.30	0.25
	20 cm.	8.13	0.21	0.93	6.00	49.9	469	3.6	4.0	45.30	6.60	4.74	11.50	1.07
	30 cm	8.13	0.21	0.96	6.22	69.3	597	3.4	3.7	44.38	5.40	4.85	11.27	3.09
2.	S2													
	10 cm.	8.00	0.44	0.74	4.99	16.6	389	3.1	3.5	45.86	0.77	9.37	9.35	9.02
	20 cm	8.06	0.30	0.86	5.41	49.5	408	3.3	3.6	45.79	0.35	4.77	8.04	3.58
	30 cm	8.06	0.29	0.80	5.35	16.2	314	3.0	3.4	43.98	0.33	3.14	8.25	3.70
3.	S3													
	10 cm.	8.05	0.31	0.77	5.09	17.5	501	3.5	4.0	43.41	2.09	0.88	5.43	5.08
	20 cm.	8.19	0.26	0.57	2.81	32.7	407	2.9	3.4	41.52	1.58	0.97	4.31	5.14
	30 cm.	8.17	0.23	0.53	2.56	18.8	325	3.4	3.8	37.91	1.62	1.10	5.63	4.31
	CPCB Permissible Limits	7-8.5	0-1.5	0.5-0.75		23-56	142-337				0.6	0.2	2.0	4.5

FIGURE - 4



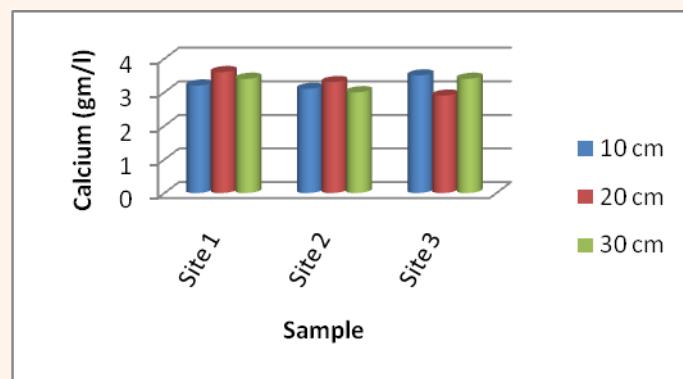
**Comparative Study of Physico-Chemical Characteristics
of Contaminated Soil**

FIGURE - 5

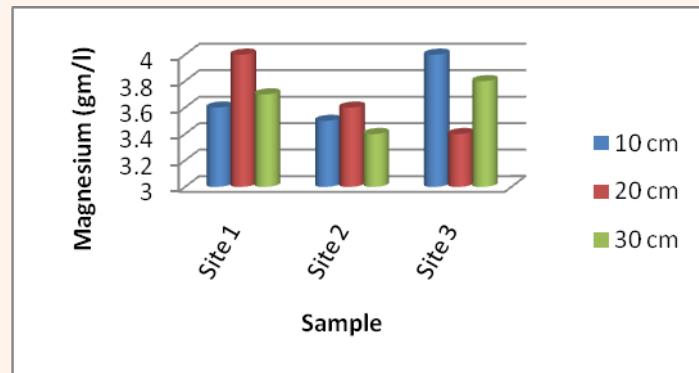


(E) Phosphorus (Kg/ha.)

(F) Potassium (Kg/ha.)



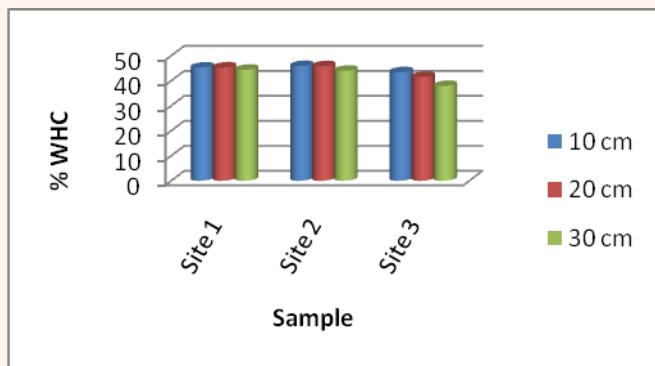
(G) Calcium (gm/l)



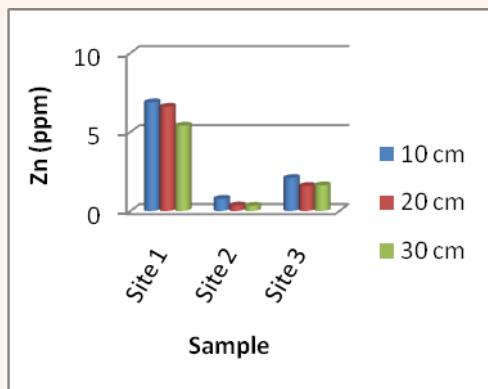
(H) Magnesium (gm/l)

**Comparative Study of Physico-chemical Characteristics
of Contaminated Soil**

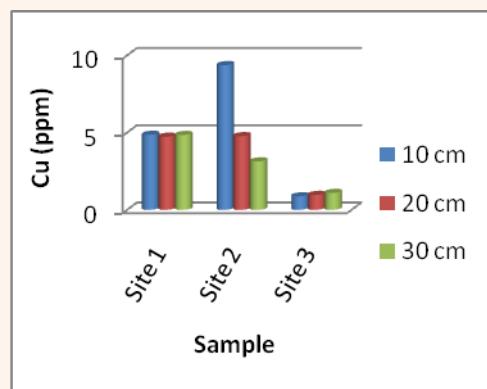
FIGURE - 6



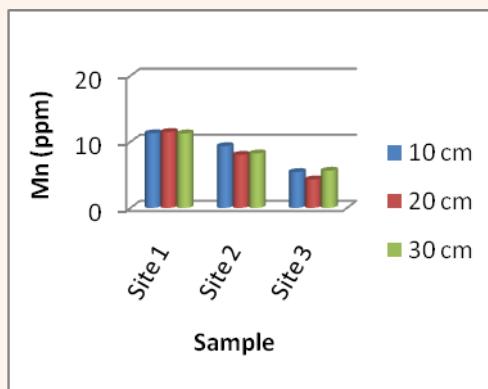
(I) % Water Holding Capacity



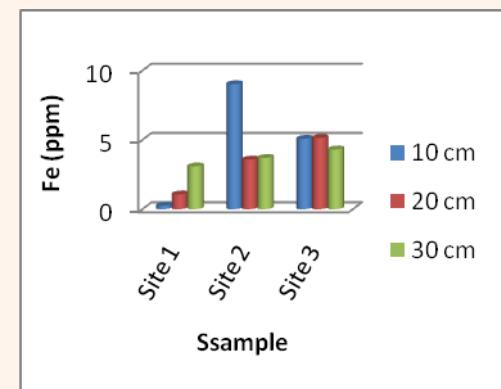
(J) Zinc (ppm)



(K) Copper (ppm)



(L) Manganese (ppm)



(M) Iron (ppm)

Comparative Study of Physico-chemical Characteristics of Contaminated Soil

PLATE - 7



(A) Plant with flower



(B) Pods of Plants



(C) Root system in Plants

Various parts of both the Experimental Plants

4.3: Effect of dyeing and printing effluent on germination and seedling growth of *Glycine max L.*: (Petri plate Method)

The results of present research work reveals that higher concentrations of dyeing effluent adversely influence the seed germination and seedling growth of experimental plants. Germination study on seeds of the experimental plants i.e. *Glycine max L.* var. JS-335 has been done on 14th day after sowing (DAS).

Germination and seedling growth in Glycine max L.

The effects of effluent on germination and seedling growth of *Glycine max L.* are shown in Table 8 and Figure 7. The seeds of *Glycine max L.* germinate in control on 2nd day of sowing and 93% seeds were germinate within 7 days (168 hours). The seeds pre-soaked with different treatment levels of the dyeing and printing effluent showed different effect, depending upon the concentrations used. Seeds pre-soaked in T₁ (20%) treatment attained maximum germination (96%) on 7th day after sowing, which is higher than control (93%). At T₂ (40%) treatment level 70% seeds were germinate on 7th day while seeds pre-soaked in increasing concentration of treatment level T₃ (60%), T₄ (80%) showed delayed and inhibited germination i.e. 56% and 40% respectively followed by T₅ (100%) where 33% seed germination was observed. In the present study no morality was observed in all the treatments and treated seeds were exhibited a successful germination. This study reveals that the dyeing and printing effluent was not significantly affecting the seed germination.

The highest value of vigour index was observed at T₁ (20%) treatment level i.e. 1680, followed by control 1590. Vigour index values of *Glycine max L.* was 1204, 856.8, 436 obtained at T₂ (40%), T₃ (60%) and T₄ (80%) levels whereas lowest vigour index value was obtained at T₅ (100%) treatment level i.e. 234.3 (Table 8 and Figure 7).

The shoot length of the seedlings of *Glycine max L.*, varied from 12.8 cm to 5.2 cm. The values of shoot length were same at control and T₂ (40%) treatment level i.e 12.6 cm. While at T₁ treatment level (20%) shoot length was 12.8cm which is slightly increased (1.5%) in comparison to control. At treatment level T₃ (60%)

Observation and Results

and T₄ (80%) the shoot length of seedling was 11.9 cm and 8.8 cm respectively whereas minimum shoot length was observed at T₅ (100% raw) effluent treatment level i.e. 5.2 cm. which is 58.7% reduced in comparison to control (Table 8 and Figure 7).

The root length of seedling was also reduced with the increasing concentrations of effluent. At T₁ treatment level where effluent was diluted 80% by distilled water root length was 4.7 cm which is higher than control 4.5 cm, root length of seedling was 4.7 cm. No significant changes were found at T₂ treatment level (4.6 cm.) in comparison to control. A gradual decline was observed in root length of seedlings from T₃ to T₅ treatment level. At T₃ (60%) and T₄ (80%) treatment level values of root length were recorded 3.4 cm. and 2.1 cm. followed by T₅ (100%) treatment level i.e. 1.9 cm (Table 8 and Figure 7).

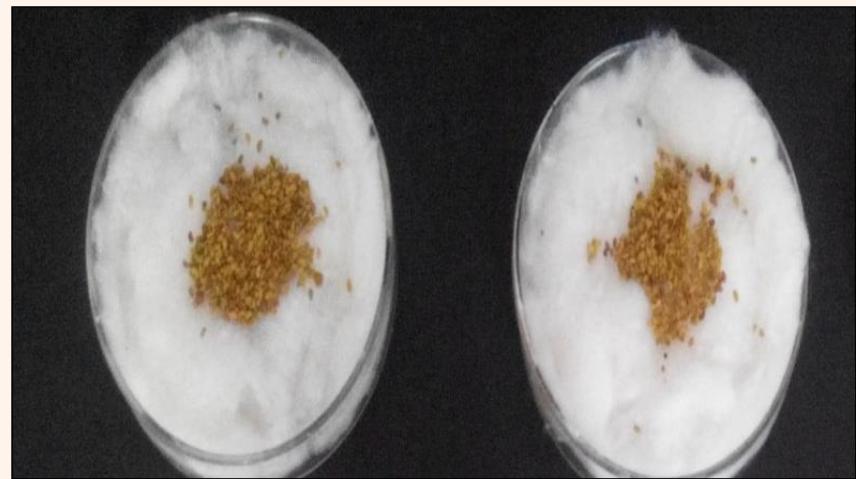
In case of fresh weight of plant *Glycine max* L. minimum value was observed in T₅ (100% raw effluent) treatment level i.e. 2.10 gm while maximum value of fresh weight was found in T₁ (20%) i.e. 3.26 gm followed by T₂ (40%) treatment level 3.15 gm. At control fresh weight of plant was 3.10 gm, whereas gradual decline was observed in fresh weight values at treatment level T₃ (60%) and T₄ (80%) i.e. 2.98 gm and 2.42 gm. Dry weight of plant *Glycine max* L. varied from 1.98 gm to 1.09 gm when they were treated with 20% to 100% dyeing effluent. Maximum value of dry weight (1.98 gm) was observed at T₁ treatment level (20%) which is 9.2% increase in comparison to control 1.90 gm. The minimum dry weight (1.09 gm) of plant was recorded at T₅ (100%) treatment level which is 42.6% decreased in comparison to control (Table 8 and Figure 8).

The pigment contents viz. Chlorophyll ‘a’ and Chlorophyll ‘b’ were significantly influenced by the application of different doses of dyeing and printing effluent. The maximum value of Chlorophyll ‘a’ and Chlorophyll ‘b’ was observed at T₁ treatment level where concentration of effluent was 20%. At T₁, Chlorophyll ‘a’ and Chlorophyll ‘b’ were 5.11 mg/g and 5.07 mg/g respectively i.e. 0.98% and 0.99% higher than control (5.06 mg/g and 5.02 mg/g) respectively. A gradual decline was observed from T₃ to T₅ level.

PLATE - 8



(A) Sterilized seeds of *Glycine max* L.



(B) Sterilized seeds of *Medicago sativa* L.

Seeds of Selected Legume Plants for Study

Observation and Results

Table 8: Effect of dyeing and printing effluent on germination and seedling growth of *Glycine max L.* (Petri plate Method)

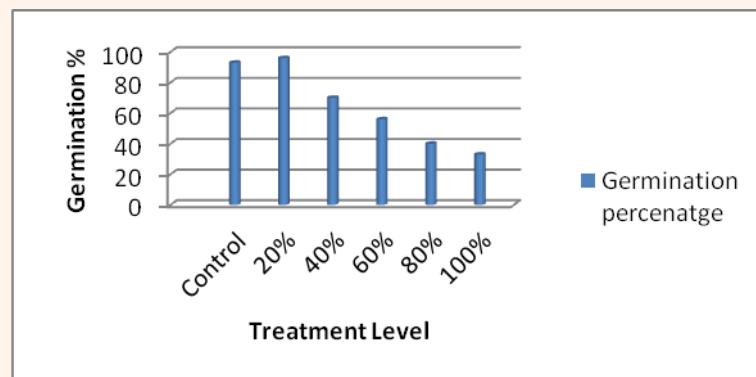
(Values are Mean±Standard Deviation of 3 replicates)

S. No.	Treatment Level	Germination Percentage	Vigour Index	Seedling growth (14 th days)		Weight		Chlorophyll content (mg/gm tissue)	
				Shoot Length (cm)	Root Length (cm)	Fresh Weight (gm)	Dry Weight (gm)	Chlorophyl a (mg/gm)	Chlorophyll b (mg/gm)
1.	Control	93	1590±0.013	12.6±0.012	4.5±0.021	3.10±0.023	1.90±0.015	5.06	5.02
2.	T ₁	96	1680±0.026 (+5.4%) **	12.8±0.020 (+1.5%) **	4.7±0.020 (+4.4%) **	3.26±0.022 (+5.1%) **	1.98±0.020 (+9.2%) **	5.11±0.021 (0.98%) **	5.07±0.019 (0.99%) **
3.	T ₂	70	1204±1.001 (+24.2%) *	12.6±0.011	4.6±0.016 (+2.2%)**	3.15±0.026 (+1.5%) **	1.93±0.021 (+1.5%) **	5.08±0.015 (+0.39%) **	5.05±0.011 (+0.59%) **
4.	T ₃	56	856.8±0.020 (-46.1%) *	11.9±0.025 (-5.5%)*	3.4±0.011 (-24.4%)**	2.98±0.013 (-3.8%)*	1.51±0.035 (-20.5%)*	4.86±0.054 (-3.95%) *	3.71±0.024 (-26.09%) *
5.	T ₄	40	436±0.017 (-72.5%)*	8.8±0.015 (-30.1%)*	2.1±0.010 (-45.3%)*	2.42±0.020 (-21.9%)*	1.26±0.013 (-33.6%)*	3.87±0.017 (-23.5%) *	2.69±0.031 (-46.4%) *
6.	T ₅	33	234.3±0.011 (-85.2%)*	5.2±0.021 (-58.7%)*	1.9±0.011 (-57.7%)*	2.10±0.011 (-32.2%)*	1.09±0.010 (-42.6%)*	2.42±0.024 (-52.1%) *	1.80±0.011 (-64.1%) *

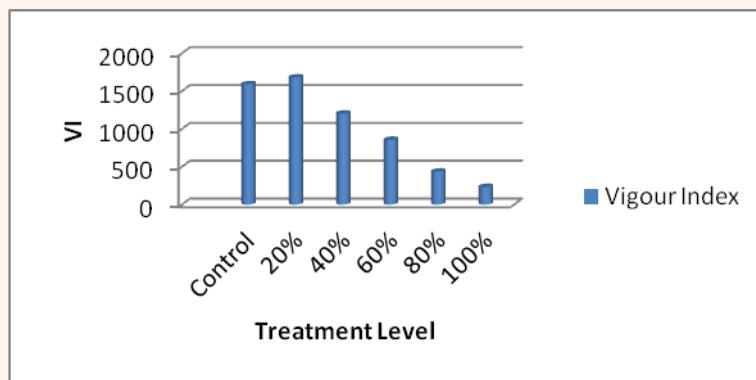
*Figures in parentheses represent % decrease over control.

**Figures in parentheses represent % increase over control.

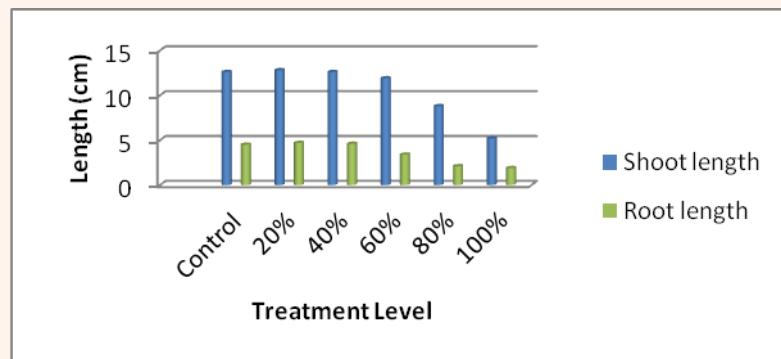
FIGURE - 7



(A) Germination Percentage



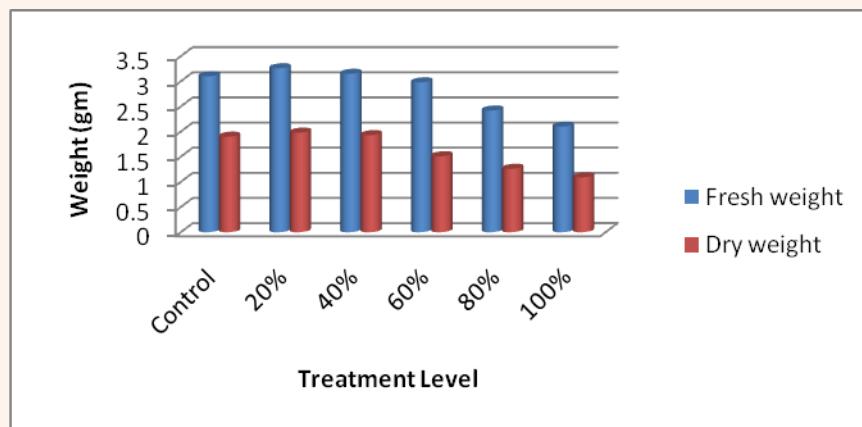
(B) Virour Index



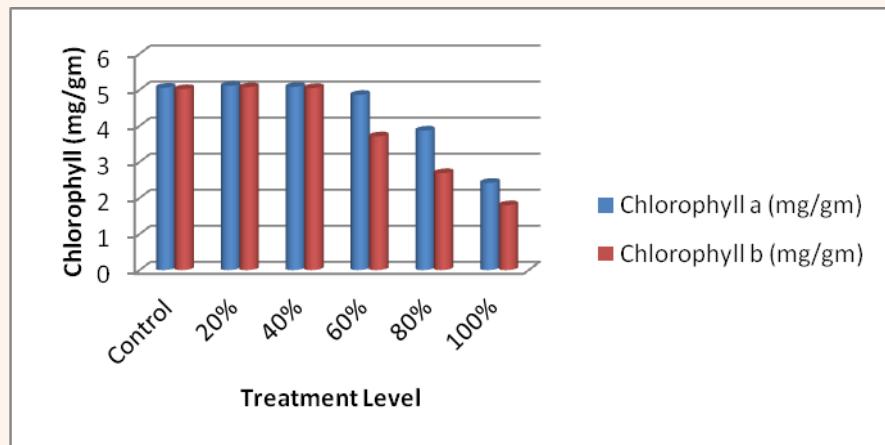
(C) Shoot length and Root length

Graph showing Effect of different treatment levels of dyeing and printing effluent on various growth parameters in *Glycine max L.* (Petri plate Method)

FIGURE - 8



(D Fresh and Dry weight (gm)



(E) Chlorophyll Content (mg/gm)

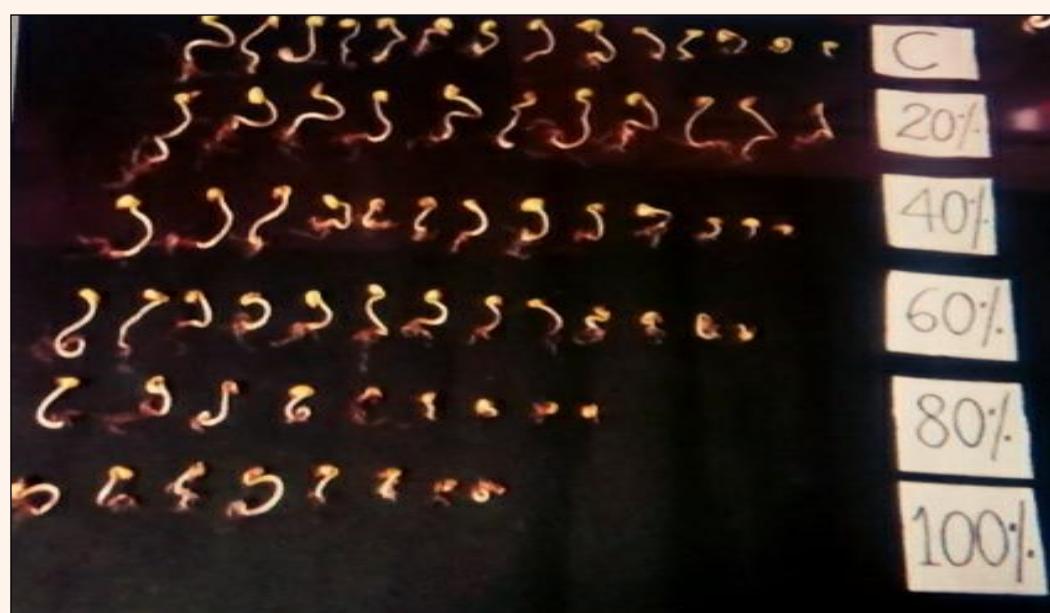
Graph showing Effect of different treatment levels of dyeing and printing effluent on various growth parameters in *Glycine max L.* (Petri plate Method)

PLATE - 9

Control 20% 40% 60% 80% 100%



(A) Percentage Germination



(B) Percentage Germination

Showing Percentage Germination of *Glycine max* L.
in Different Treatment Levels of Dyeing and Printing Effluent
(Petri-Plate Method)

Maximum reduction in Chlorophyll ‘a’ and Chlorophyll ‘b’ i.e. 2.42 mg/g and 1.80 mg/g were recorded at T₅ treatment level (100% raw effluent). The pigment content were significantly affected by the effluent, at T₅ treatment level (raw effluent) Chlorophyll ‘a’ and Chlorophyll ‘b’ were decreased 52.1% and 64.1% respectively in comparison to control (Table 8 and Figure 8).

4.4: Evaluation of various growth and biochemical parameters of *Glycine max L.* treated with different concentration of dyeing and printing effluent: (Pot Experiment)

Figure 9, 10, 11, 12, 13, 14 shows the relative degree of enhancement or inhibition on seedling growth (Shoot and Root growth, fresh and dry weight of shoot and root, vigour index and N, P, K Content in seedling), productivity, pigment content, biochemical parameters and nodulation. The effluent has significant effect on seed germination in early stage which has gradually decreased. Higher concentration (60%, 80% and 100%) of effluent had inhibitory effect than that of lower concentration (20% and 40%). Various growth parameters were observed twice, which was on 30 day of sowing and after the completion of life cycle of the experimental plant i.e. *Glycine max L.*

4.4.1 Effect on Growth and Pigment content of *Glycine max L.* after 30 day of sowing

(A) Shoot length

A gradual decline in shoot length of *Glycine max L.* was observed with increasing concentration of dyeing and printing effluent (Table 9, Figure 9). At 30 DAS, the average highest shoot length was recorded in T₁ (20%) treatment level (30.56 cm), followed by T₂ (40%) treatment level (29.46 cm) and control (27.33 cm). At T₁ and T₂ treatment level shoot length was increased 11.81% and 7.79% respectively as compare to control. Higher concentration of effluent had maximum inhibitory effect i.e. T₅ (100%) treatment level, where shoot length was 20.5cm which is 24.99% reduced in comparison to control.

Observation and Results

(B) Root length

The root length of *Glycine max* L. on 30 day of sowing varied from 18.73 cm to 10.8 cm. The maximum root length was observed at T₁ treatment level i.e. 18.73 followed by T₂ level i.e. 16.2 cm. The root length was 22.98% and 6.63% enhanced at T₁ and T₂ treatment level respectively in comparison to control i.e. 15.23 cm. The inhibition of root length was observed at T₃, T₄ and T₅ treatment level i.e. 13.2 cm, 11.56 cm and 10.8 cm respectively which were 13.32%, 24.09% and 29.08% respectively reduce as compared with control as shown in Table 9, figure 9.

(C) Fresh Weight of Shoot

Table 9 and Figure 10 illustrate the relative degree of enhancement or inhibition by effluent on fresh weight and dry weight of shoots and roots of *Glycine max* L. In case of fresh weight of shoots in *Glycine max* L., minimum value was observed on 30 days at treatment level T₅ (100% raw effluent) i.e. 13.2 gm/plant which was 14.28% decreased in comparison to control while maximum value of fresh weight of shoot was observed at T₁ (20%) and T₂ (40%) treatment level i.e. 17.2 gm/plant and 17.0 gm/plant which was 11.68% and 10.38% respectively increased when compared with control (15.4 gm/plant).

(D) Fresh Weight of Roots

Maximum fresh weight of plant roots treated with dyeing and printing effluent was found in treatment level T₁ (20%) and T₂ (40%) (12.73 gm/plant and 11.8 gm/plant respectively) which were 20.89% and 12.06% increased as compared with control (10.53 gm/plant). The fresh weight of root was decreased with the increasing concentration of effluent where fresh weight of root was 10.13 gm/plant, 9.6 gm/plant and 8.83 gm/plant at T₃, T₄ and T₅ treatment level. Maximum reduction in fresh weight of root was at T₅ treatment level where fresh weight was decreased 16.14% as compared with control (Table 9, Figure 10).

Observation and Results

(E) Dry Weight of Shoots

The highest dry weight of shoot was recorded (Figure 9, Table 6) at T₁ and T₂ treatment levels i.e. 4.13 gm/plant and 3.7 gm/plant which were slightly increased over control (3.53 gm/plant). The relative percentage inhibition in dry weight of shoot was recorded from treatment level T₃ (3.68%) > T₄ (11.33%) > T₅ (16.14%) when compared with control (Table 9, Figure 10).

(F) Dry Weight of Roots

At 30 days after sowing Figure 9 and Table 6 illustrate the relative degree of enhancement or inhibition by effluent on dry weight of roots. Dry weight of roots was relatively enhanced by 20% (T₁) that showed maximum value of dry weight of root i.e. 2.4 gm/plant followed by T₂ treatment level i.e. 2.26 gm/plant. At T₁ treatment level dry weight of root was increased 31.14% over control. Maximum reduction in dry weight of root was recorded at T₅ treatment level (1.08 gm/plant) which is 40.98% reduced when compared with control. The relative percentage inhibition in dry weight of root was found 21.85% at 60% effluent concentration and 32.78% at 80% of effluent concentration (Table 9, Figure 10).

(G) Vigour Index

The vigour index varied from 4354.66 to 1462.78 between control to T₅ treatment level. The vigour index was decrease with the increasing concentration of effluent. The minimum value of vigour index was found at T₅ (100%) treatment level i.e. 1462.78 which was 56.12% decreased as compared with control 3333.81. At T₁ (20%) and T₂ (40%) treatment level vigour index value of *Glycine max L.* plant was higher than control. The percentage increase was 30.62% and 14.14% over control at T₁ and T₂ treatment level respectively (Table 9, Figure 11).

(H) Pigment content

The chlorophyll and carotenoid content in the experimental plant was studied and results are presented in Table 11, Figure 12. Increase level of pigment content was recorded when effluent was diluted 80% with the normal water i.e. T₁ treatment level. The chlorophyll ‘a’ and chlorophyll ‘b’ was found maximum at T₁ treatment level i.e. 6.08 mg/gm fresh weight and 5.15 mg/gm respectively which were 39.44% and 20.61% higher than control 4.36 mg/gm fresh weight and 4.27 mg/gm fresh weight respectively. A gradual decline was observed with the higher concentration of effluent but at T₂ treatment level chlorophyll ‘a’ was 16.75% enhance when compared with control 4.36 mg/gm fresh weight. The minimum value of chlorophyll ‘a’ and chlorophyll ‘b’ was found at T₅ treatment level (100% raw effluent) i.e. 2.78 mg/gm fresh weight and 2.62 mg/gm fresh weight respectively. Chlorophyll ‘a’ and chlorophyll ‘b’ reduced 36.23% and 38.64% respectively at T₅ treatment level when compared with control. It was observed that effect of highly diluted effluent on total chlorophyll content in plant shows stimulatory effect rather than inhibitory effect in T₁ (13.43 mg/gm) treatment level followed by control 9.71 mg/gm and percentage increase was found 38.31% over control. While highly reducing value of total chlorophyll content was found in treatment level T₅ (8.87 mg/gm) which was 8.65% decreased as compared with control. A gradual decreasing values in increasing concentration of effluent at treatment level T₂, T₃ and T₄ i.e. 9.61 mg/gm fresh weight, 9.41 mg/gm fresh weight and 9.27 mg/gm fresh weight respectively. Reduction in percentage was increased from T₂ to T₄ i.e. 2.05%, 3.08% and 5.53% respectively in comparison to control. The values of carotenoids varied from 3.63 mg/gm fresh weight to 1.61 mg/gm fresh weight when treated with various concentrations of effluent. Maximum carotenoid content was recorded at T₁ treatment level (3.63 mg/gm fresh weight) that was 19.01% enhance over control (3.01 mg/gm fresh weight). The minimum value of carotenoid content was at T₅ treatment level (1.61 mg/gm fresh weight) which was 47.21% decreased as compared with control.

4.4.2 Effect on Growth and yield of *Glycine max* L. after completion of life cycle (60 DAS)

(A) Shoot length

The result showed (Table 10, Figure 9) that plant growth of *Glycine max* L. was influenced by different concentrations of dyeing and printing effluent. At 60 DAS, the average highest shoot length was recorded in T₁ (20%) treatment level (37.76 cm), followed by T₂ (40%) treatment level (36.4 cm) and control (35.86 cm). At T₁ and T₂ treatment level shoot length was increased 5.29% and 1.50% respectively as compare to control. Higher concentration of effluent had maximum inhibitory effect i.e. at T₅ (100%) treatment level, where shoot length was 20.34cm which is 43.27% reduced in comparison to control. At T₃ and T₄ treatment level shoot length was reduced 27.44% and 33.88% when compared with control.

(B) Root length

A gradual decline was observed in root length with the increasing concentration of the effluent. The root length of *Glycine max* L. varied from 25.12 cm to 16.72 cm. The maximum root length was observed at T₁ treatment level i.e. 25.12 cm followed by T₂ level i.e. 23.95 cm. The root length was 10.36% and 5.22% enhanced at T₁ and T₂ treatment level respectively in comparison to control i.e. 22.76 cm. The inhibition of root length was observed at T₃, T₄ and T₅ treatment level i.e. 20.63 cm, 18.14 cm and 16.72 cm respectively which were 9.35%, 20.29% and 26.53% respectively reduce as compared with control (Table 10, Figure 9).

(C) Fresh Weight of Shoot

Maximum fresh weight of plant shoot treated with dyeing and printing effluent was found in treatment level T₁ (20%) and T₂ (40%) (20.72 gm/plant and 18.02 gm/plant respectively) which were 11.09% and 5.73% increased when compared with control (18.09 gm/plant). The fresh weight of shoot was decreased with the increasing concentration of effluent where fresh weight of shoot was 16.4

Observation and Results

gm/plant, 14.06 gm/plant and 11.14 gm/plant at T₃, T₄ and T₅ treatment level. Maximum reduction in fresh weight of shoot was at T₅ treatment level where fresh weight was decreased 20.26% as compared with control (Table 10, Figure 10).

(D) Fresh Weight of Roots

A gradual decline in fresh weight of roots was observed with increasing concentration of effluent (Table 10, Figure 10). Minimum value of fresh weight of roots in *Glycine max* L., was observed at treatment level T₅ (100% raw effluent) i.e. 7.49 gm/plant which was 32.21% decreased in comparison to control while maximum value of fresh weight of root was observed at T₁ (20%) and T₂ (40%) treatment level i.e. 13.62 gm/plant and 11.82 gm/plant which was 23.25% and 6.96% respectively increased when compared with control (11.05 gm/plant).

(E) Dry Weight of Shoot

Dry weight of shoot was ranged between 4.86 gm/plant to 3.03 gm/plant. Observations showed maximum values at 20% (T₁) effluent concentration i.e. 4.86 gm/plant which is 11.56% increased as compared with control 4.46 gm/plant. The dry weight of shoot inhibit when plants treated with higher concentration of effluent. Reduction in percentage increased from T₃ to T₅ i.e. 19.07%, 26.01% and 41.32% respectively (Table 10, Figure 10).

(F) Dry Weight of Roots

Dry weight of root of *Glycine max* L. varied from 1.86 gm/plant to 2.76 gm/plant. Maximum dry weight of root was recorded at T₁ treatment level i.e. 2.76 gm/plant which increased 18.45% than control 2.33 gm/plant. The maximum reduction in percentage was observed at T₅ treatment level i.e. 19.74% when compared with control. Dry weight of root was approximately equal at T₃ and control (Table 10, Figure 10).

(G) Vigour Index

The vigour index varied from 5834.56 to 1832.24 at control and T₁ to T₅ treatment level (Table 10, Figure 11). The vigour index was decrease with the increasing concentration of effluent. The minimum value of vigour index was found at T₅ (100%) treatment level i.e. 1832.24 which was 63.00% decreased as compared with control 4952.91. At T₁ (20%) and T₂ (40%) treatment level vigour index value of *Glycine max* L. plant was higher than control. The percentage increase was 17.80% and 7.25% over control at T₁ and T₂ treatment level respectively.

(H) N, P K Content

A gradual decline was observed in N, P, K Content with the increasing concentration of the effluent. In *Glycine max* L. Nitrogen content varied from 5.28% to 2.38%, Phosphorus content varied from 4.60% to 1.42% and Potassium content varied from 4.62% to 2.57%. The maximum Nitrogen content was observed at T₁ treatment level i.e. 5.28% followed by T₂ level i.e. 4.81%. The Nitrogen content was 26.31% and 15.07% enhanced at T₁ and T₂ treatment level respectively in comparison to control i.e. 4.18%. The inhibition of Nitrogen content was observed at T₃, T₄ and T₅ treatment level i.e. 2.66%, 2.51% and 2.38% respectively which were 36.36%, 39.95% and 43.06% respectively reduce as compared with control. The maximum Phosphorus content was observed at T₁ treatment level i.e. 4.60% followed by T₂ level i.e. 2.24%. The Phosphorus content was 14.71% and 2.24% enhanced at T₁ and T₂ treatment level respectively in comparison to control i.e. 4.01%. The inhibition of Phosphorus content was observed at T₃, T₄ and T₅ treatment level i.e. 3.73%, 2.55% and 1.42% respectively which were 6.98%, 36.40% and 64.58% respectively reduce as compared with control. Similarly the maximum Potassium content was observed at T₁ treatment level i.e. 4.62% followed by T₂ level i.e. 3.28%. The Potassium content was 43.47% and 1.83% enhanced at T₁ and T₂ treatment level respectively in comparison to control i.e.

Observation and Results

4.01%. The inhibition of Potassium content was observed at T₃, T₄ and T₅ treatment level i.e. 3.05%, 3.02% and 2.57% respectively which were 5.27%, 6.21% and 20.18% respectively reduce as compared with control (Table 10, Figure 11).

(I) Yield Parameters

The productivity of the treated plant (*Glycine max L.*) was also affected with the increased concentration of dyeing and printing effluent (Table 13, Figure 13). The yield parameters viz. pod per plant, weight of pod per plant, no. of seeds per pod and weight of seed per pod were recorded after the completion of life cycle. The maximum pods/plant was observed at T₁ treatment level followed by T₂ and control. At T₁ and T₂ treatment level pods/plant was 11.66 and 9.66 which was 34.64% and 11.54% higher than control (8.36). The minimum pods/plant was observed at T₅ treatment level i.e. 5.33. The reduction in percentage was 38.45% when compared with control. A gradual decline in pods weight/plant of *Glycine max L.* was observed with increasing concentration of dying and printing effluent. The average highest pods weight/plant was recorded in T₁ (20%) treatment level (5.5 gm), followed by T₂ (40%) treatment level (4.46 gm) and control (4.13 gm). At T₁ and T₂ treatment level pods weight/plant was increased 33.17% and 7.99% respectively as compare to control. Higher concentration of effluent had maximum inhibitory effect i.e. T₅ (100%) treatment level, where pods weight/plant was 3.06 gm which is 25.90% reduced in comparison to control.

The seed/pod was higher at T₁ treatment level (3.33) and at T₂ treatment level (2.66) than that of control (2.33). Increase in effluent concentration from 60% onwards reduced the no. of seeds per pod. The minimum no. of seeds/pod (1.33) was found at T₅ treatment level that was 42.91% reduced when compared with control. Maximum seed weight/pod treated with dyeing and printing effluent was found in treatment level T₁ (20%) and T₂ (40%) (5.43 gm and 3.83 gm respectively) which were 50.83% and 6.38% increased as compared with control (3.6 gm). The seed weight/pod was decreased with the increasing concentration of effluent where

Observation and Results

seed weight/pod was 2.98 gm, 1.99 gm and 1.71 gm at T₃, T₄ and T₅ treatment level. Maximum reduction in seed weight/pod was at T₅ treatment level where seed weight was decreased 52.5% as compared with control.

4.4.3: Effect on biochemical parameters of *Glycine max L.*

Table 12, 14 and Figure 12, 13 shows the effect of effluent on chlorophyll ‘a’, chlorophyll ‘b’, carotenoids, proteins, free amino acids and free sugar.

4.4.3.1 Pigment content

The pigment content was also recorded after the completion of life cycle of the treated *Glycine max L* (Table 12, Figure 12) plant. Results showed that the value of chlorophyll ‘a’, chlorophyll ‘b’ and carotenoid content was higher when plants treated with 20% and 40% concentrated effluent. At T₁ chlorophyll ‘a’ and chlorophyll ‘b’ was 8.69 mg/gm fresh weight and 8.44 mg/gm fresh weight followed by T₂ (6.86 mg/gm fresh weight and 6.48 mg/gm fresh weight respectively) and control (6.01 mg/gm fresh weight and 5.32 mg/gm fresh weight respectively). A decreasing pattern was observed in the values of all the pigment content with the increasing concentration of effluent from T₃ to T₅ (60% to 100%). The maximum reduction in chlorophyll ‘a’ and ‘b’ was found at T₅ treatment level (3.93 mg/gm fresh weight and 3.87 mg/gm fresh weight respectively) which were 34.60% and 21.64% reduced when compared with control. Total chlorophyll content in *Glycine max L.* was also adversely affect with the increasing concentration of effluent. The values of carotenoids varied from 4.22 mg/gm fresh weight to 1.29 mg/gm fresh weight when treated with various concentrations of effluent. Maximum carotenoid content was recorded at T₁ treatment level (4.22 mg/gm fresh weight) that was 9.04% enhance over control (3.87 mg/gm fresh weight). The minimum value of carotenoid content was at T₅ treatment level (1.29 mg/gm fresh weight) which was 66.66% decreased as compared with control (Table 14, Figure 13).

4.4.3.2: Protein

The protein content in *Glycine max* L. was negatively affected when treated with higher concentration of dyeing and printing effluent (Table 14, Figure 13). As effluent concentration increased from T₃ (60%) to T₅ (100%) the protein content was adversely affected. The maximum reduction was found at T₅ (100% effluent) treatment level i.e. 7.22 mg/gm fresh weight which decreased 45.34% as compared with control. Increased protein content was recorded at T₁ treatment level i.e. 13.91 mg/gm fresh weight which enhance 5.29% over control (13.21 mg/gm fresh weight). The value of protein was approximately similar in plants treated with 40% effluent (13.22 mg/gm fresh weight) and control.

4.4.3.3: Free Amino acid

Glycine max L. plants when treated with various concentration of dyeing and printing effluent the free amino acid ranged between 10.45 mg/gm fresh weight to 2.52 mg/gm fresh weight. Highest amount (10.45 mg/gm fresh weight) of free amino acid was observed at T₁ treatment level which was 43.93% increased over control. A gradual decline in free amino acid content was observed with the increasing concentration of effluent. At T₃, T₄ and T₅ the free amino acid was 5.08 mg/gm fresh weight, 3.03 mg/gm fresh weight, 2.52 mg/gm fresh weight respectively which were 30.02%, 58.26% and 65.28% reduced when compared with control (7.26 mg/gm fresh weight). The difference in the value of free amino acid at T₂ (7.14 mg/gm fresh weight) and control is negligible (Table 14, Figure 13).

4.4.3.4: Free Sugar

Free sugar in treated plant was also adversely affected with the increasing concentration of effluent. The maximum (12.08 mg/gm fresh weight) free sugar content was recorded at T₁ treatment level which was 8.06% higher when compared with control 11.18 mg/gm fresh weight. At T₂ treatment level also free sugar content was 3.56% increased over the control. The minimum (10.40 mg/gm fresh weight) free sugar content was recorded at T₅ treatment level which was 6.99% decreased when compared with control (Table 14, Figure 13).

Observation and Results

Table 9: Effect of dyeing and printing effluent on seedling growth of *Glycine max L.* after 30 day of sowing

(Values are mean \pm Standard Deviation of 3 replicates)

S. No.	Treatment Level	Shoot Length (cm)	Root Length (cm)	Shoot Fresh Weight (gm/plant)	Root Fresh Weight (gm/plant)	Shoot Dry Weight (gm/plant)	Root Dry Weight (gm/plant)	Vigour Index
1.	Control	27.33 \pm 0.032	15.23 \pm 0.035	15.4 \pm 0.043	10.53 \pm 0.035	3.53 \pm 0.035	1.83 \pm 0.025	3333.81 \pm 4.213
2.	T ₁	30.56 \pm 0.030 (+11.81%)**	18.73 \pm 0.020 (+22.98%)**	17.2 \pm 0.03 (+11.68%)**	12.73 \pm 0.040 (+20.89%)**	4.13 \pm 0.040 (+16.99%)**	2.4 \pm 0.043 (+31.14%)**	4354.66 \pm 4.486 (+30.62%)**
3.	T ₂	29.46 \pm 0.035 (+7.79%)**	16.2 \pm 0.026 (+6.36%)**	17 \pm 0.026 (+10.38%)**	11.8 \pm 0.03 (+12.06%)**	3.7 \pm 0.052 (+4.81%)**	2.26 \pm 0.035 (+23.49%)**	3805.4 \pm 4.301 (+14.14%)**
4.	T ₃	25.83 \pm 0.025 (-5.48%)*	13.2 \pm 0.04 (-13.32%)**	14.93 \pm 0.015 (-3.05%)*	10.13 \pm 0.045 (-3.79%)*	3.4 \pm 0.043 (-3.68%)*	1.43 \pm 0.020 (-21.85%)*	2667.14 \pm 5.308 (-19.99%)*
5.	T ₄	23.2 \pm 0.045 (-15.11%)*	11.56 \pm 0.030 (-24.09%)*	14.06 \pm 0.025 (-8.70%)*	9.6 \pm 0.043 (-8.83%)*	3.13 \pm 0.025 (-11.33%)*	1.23 \pm 0.005 (-32.78%)*	1910.33 \pm 3.987 (-42.69%)*
6.	T ₅	20.5 \pm 0.03 (-24.99%)*	10.8 \pm 0.03 (-29.08%)*	13.2 \pm 0.031 (-14.28%)*	8.83 \pm 0.030 (-16.14%)*	2.96 \pm 0.032 (-16.14%)*	1.08 \pm 0.021 (-40.98%)*	1462.78 \pm 2.541 (-56.12%)*

*Figures in parentheses represent % decrease over control.

**Figures in parentheses represent % increase over control.

Observation and Results

Table 10: Effect of dyeing and printing effluent on seedling growth of *Glycine max L.* after completion of life cycle (60 DAS)

(Values are mean \pm Standard Deviation of 3 replicates)

S. No.	Treatment Level	Shoot Length (cm)	Root Length (cm)	Shoot Fresh Weight (gm/plant)	Root Fresh Weight (gm/plant)	Shoot Dry Weight (gm/plant)	Root Dry Weight (gm/plant)	Vigour Index	N, P, K %Content in Seedling		
									N %	P %	K %
1.	Control	35.86 \pm 0.047	22.76 \pm 0.037	18.09 \pm 0.160	11.05 \pm 0.041	4.46 \pm 0.057	2.33 \pm 0.208	4952.91 \pm 6.507	4.18%	4.01%	3.22%
2.	T ₁	37.76 \pm 0.188 (+5.29%)**	25.12 \pm 0.016 (+10.36%)**	20.72 \pm 0.018 (+11.09%)**	13.62 \pm 0.021 (+23.25%)**	4.86 \pm 0.251 (+11.56%)**	2.76 \pm 0.115 (+18.45%)**	5834.56 \pm 3.791 (+17.80%)**	5.28 \pm 0.982 (+26.31%)**	4.60 \pm 0.018 (+14.71%)	4.62 \pm 0.019 (+43.47%)
3.	T ₂	36.4 \pm 0.081 (+1.50)**	23.95 \pm 0.028 (+5.22)**	18.02 \pm 0.029 (-5.73%)*	11.82 \pm 0.020 (+6.96%)**	4.43 \pm 0.2 (-2.02%)*	2.6 \pm 0.173 (+11.59%)**	5312.36 \pm 5.170 (+7.25%)**	4.81 \pm 0.105 (+15.07%)**	4.10 \pm 0.021 (+2.24%)	3.28 \pm 0.051 (+1.83%)
4.	T ₃	26.02 \pm 0.012 (-27.44%)*	20.63 \pm 0.012 (-9.35%)**	16.4 \pm 0.121 (-12.06%)*	10.33 \pm 0.033 (-6.51%)*	3.8 \pm 0.099 (-19.07%)*	2.3 \pm 0.026 (-1.29%)*	3370.18 \pm 5.542 (-31.95%)*	2.66 \pm 0.309 (-36.36%)*	3.73 \pm 0.124 (-6.98%)	3.05 \pm 0.009 (-5.27%)
5.	T ₄	23.71 \pm 0.009 (-33.88%)*	18.14 \pm 0.008 (-20.29%)*	14.60 \pm 0.004 (-21.71%)*	8.65 \pm 0.024 (-21.71%)*	3.56 \pm 0.030 (-26.01%)*	1.96 \pm 0.032 (-15.88%)*	2471.04 \pm 4.543 (-50.10%)*	2.57 \pm 0.916 (-39.95%)*	2.55 \pm 0.018 (-36.40%)	3.02 \pm 0.031 (-6.21%)
6.	T ₅	20.34 \pm 0.044 (-43.27%)*	16.72 \pm 0.151 (-26.53%)*	11.14 \pm 0.049 (-20.26%)*	7.49 \pm 0.072 (-32.21%)*	3.03 \pm 0.230 (-41.32%)*	1.86 \pm 0.035 (-19.74%)*	1832.24 \pm 4.212 (-63.00%)*	2.38 \pm 0.709 (-43.06%)*	1.42 \pm 0.092 (-64.58%)	2.57 \pm 0.046 (-20.18%)

*Figures in parentheses represent % decrease over control.

**Figures in parentheses represent % increase over control.

PLATE - 10



(A) *Glycine max* L. Showing Growth in Pot (i)



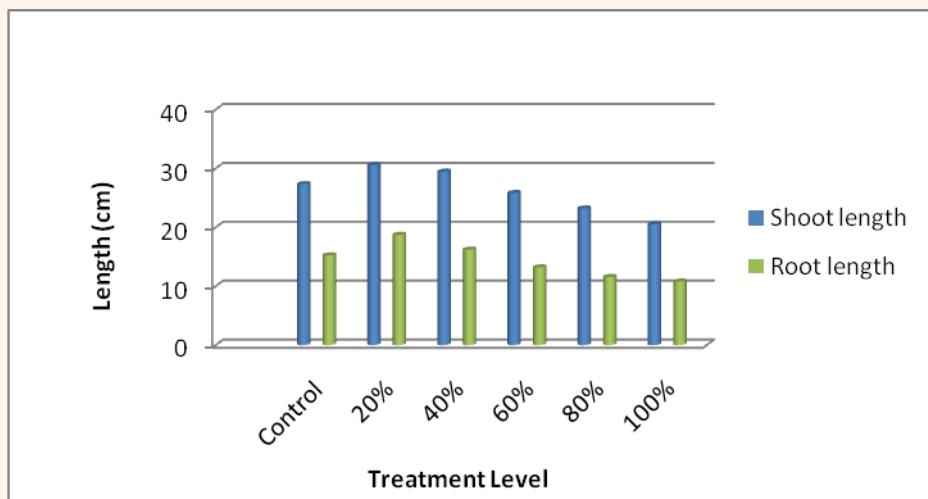
(B) *Glycine max* L. Showing Growth in Pot (ii)



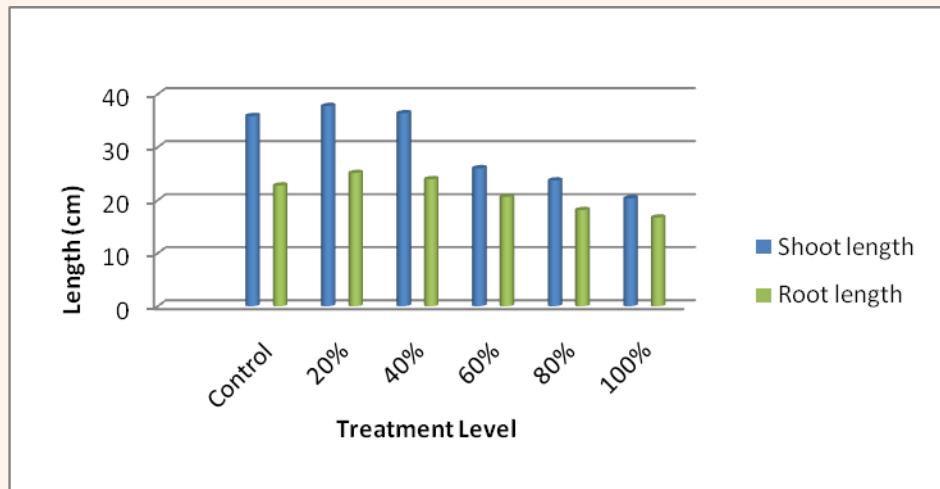
(C) *Glycine max* L. Showing Growth in Pot (iii)

Pot Experiment

FIGURE - 9



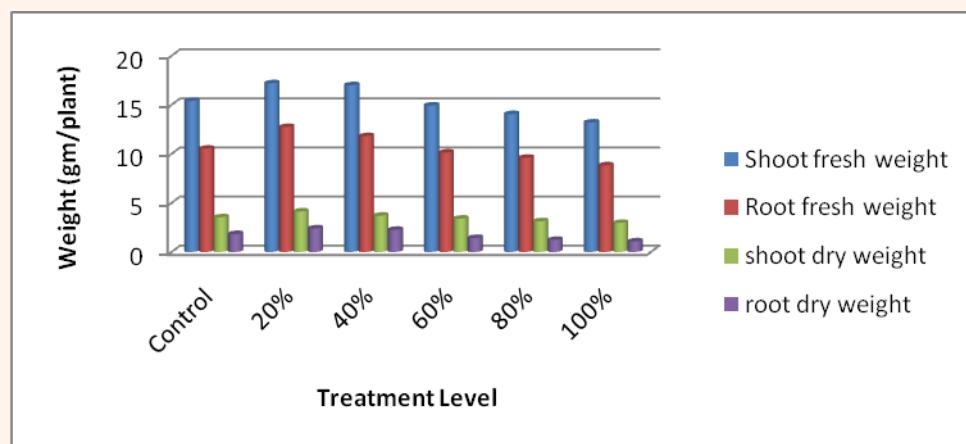
(A) Shoot and Root length (30 DAS)



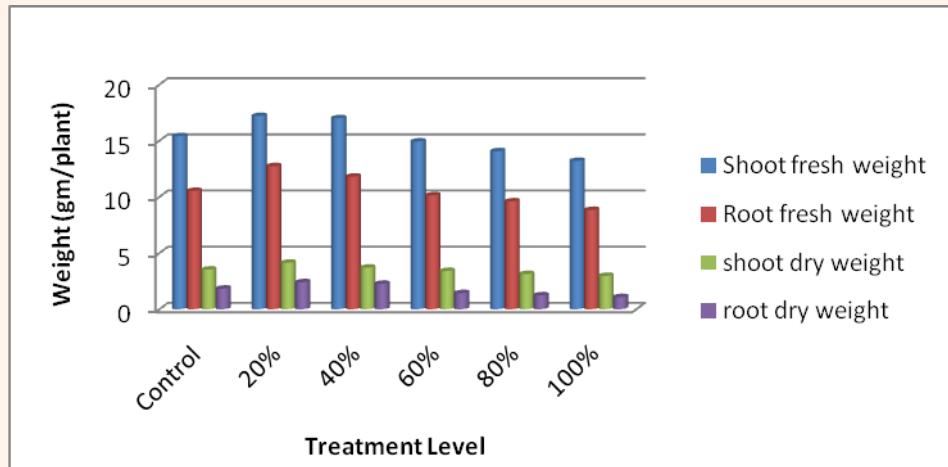
(B) Shoot and Root length (60 DAS)

Graph showing Effect of different treatment levels of dyeing and printing effluent on various growth parameters in *Glycine max L.* (Pot Experiment)

FIGURE - 10



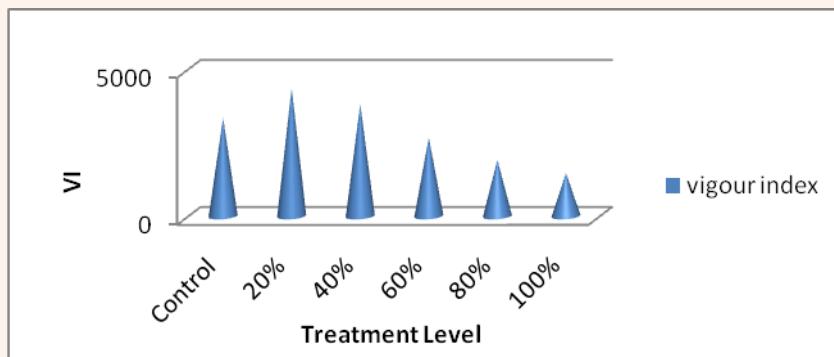
(A) Fresh and Dry Weight of Shoot and Root (30 DAS)



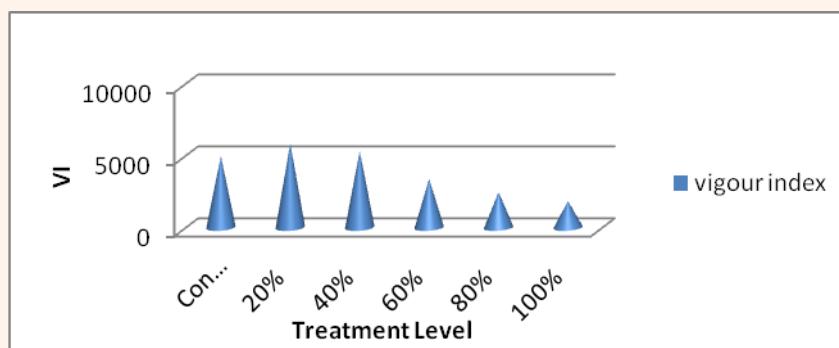
(B) Fresh and Dry Weight of Shoot and Root (60 DAS)

Graph showing Effect of different treatment levels of dyeing and printing effluent on various growth parameters in *Glycine max L.* (Pot Experiment)

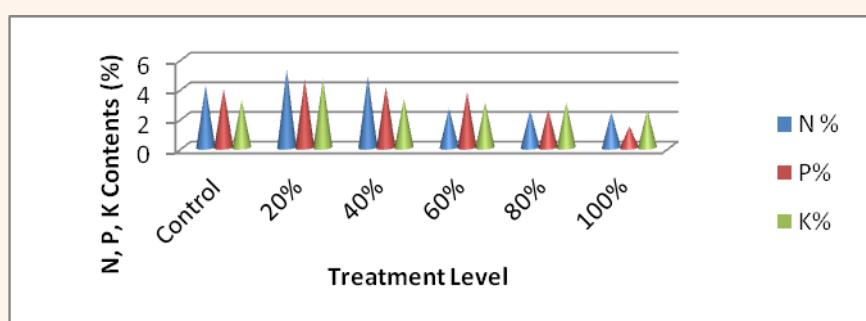
FIGURE - 11



(A) Vigour Index (30 DAS)



(B) Vigour Index (60 DAS)



(C) N, P, K (%) Contents (60 DAS)

Graph showing Effect of different treatment levels of dyeing and printing effluent on various growth parameters in *Glycine max L.* (Pot Experiment)

Observation and Results

Table 11: Effect of dyeing and printing effluent on pigment content (mg g⁻¹ fresh weight) of *Glycine max* L. after 30 day of sowing

(Values are mean ± Standard Deviation of 3 replicates)

S. No	Treatment Level	Chlorophyll a (mg/gm f.wt.)	Chlorophyll b (mg/gm f.wt.)	Total Chlorophyll (mg/gm f.wt.)	Carotenoid (mg/gm f.wt.)
1.	Control	4.36±0.024	4.27±0.032	9.71±0.036	3.05±0.012
2.	T ₁	6.08±0.09 (+39.44%)**	5.15±0.020 (+20.61%)**	13.43±0.026 (+38.31%)**	3.63±0.016 (+19.01%)**
3.	T ₂	5.09±0.04 (+16.74%)**	4.24±0.037 (-0.7%)**	9.61±0.024 (-2.05%)**	3.01±0.018 (-2.62%)**
4.	T ₃	4±0.018 (-8.25%)*	3.9±0.016 (-8.67%)*	9.41±0.012 (-3.08%)*	2.59±0.008 (-15.08%)*
5.	T ₄	2.93±0.016 (-32.79%)*	2.89±0.023 (-32.32%)*	9.27±0.024 (-4.53%)*	1.92±0.016 (-37.04%)*
6.	T ₅	2.78±0.012 (-36.23%)*	2.62±0.004 (-38.64%)*	8.87±0.016 (-8.65%)	1.61±0.035 (-47.21%)*

*Figures in parentheses represent % decrease over control.

**Figures in parentheses represent % increase over control.

Observation and Results

Table 12: Effect of dyeing and printing effluent on pigment contents (mg g⁻¹ fresh weight) of *Glycine max L.* after completion of life cycle (60DAS)

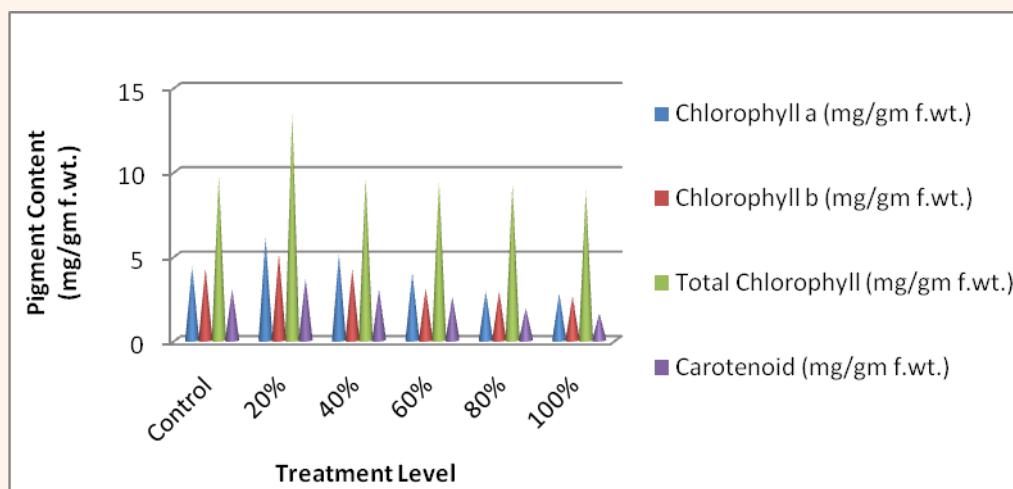
(Values are mean ± Standard Deviation of 3 replicates)

S. No	Treatment Level	Chlorophyll a (mg/gm f.wt.)	Chlorophyll b (mg/gm f.wt.)	Total Chlorophyll (mg/gm f.wt.)	Carotenoid (mg/gm f.wt.)
1.	Control	6.01±0.004	5.32±0.0264	11.33±0.015	3.87±0.056
2.	T ₁	8.69 ±0.374 (+44.59%)**	8.44±0.0608 (+45.22%)**	15.13±0.005 (+45.00%)**	4.22±0.017 (+9.04%)**
3.	T ₂	6.86±0.009 (+14.14%)**	6.48±0.0251 (+10.24%)**	13.33±0.020 (+11.54%)**	3.97±0.264 (+2.58%)**
4.	T ₃	5.12±0.021 (-14.80%)*	5.09±0.056 (-17.04%)*	10.41±0.032 (-16.84%)*	3.61±0.305 (-6.71%)*
5.	T ₄	4.76±0.012 (-20.79%)*	4.58±0.055 (-19.78%)*	10.22±0.049 (-17.94%)*	2.43±0.064 (-37.20%)*
6.	T ₅	3.93±0.016 (-34.60%)*	3.87±0.023 (-21.64%)*	9.78±0.080 (-26.25%)*	1.29±0.023 (-66.66%)*

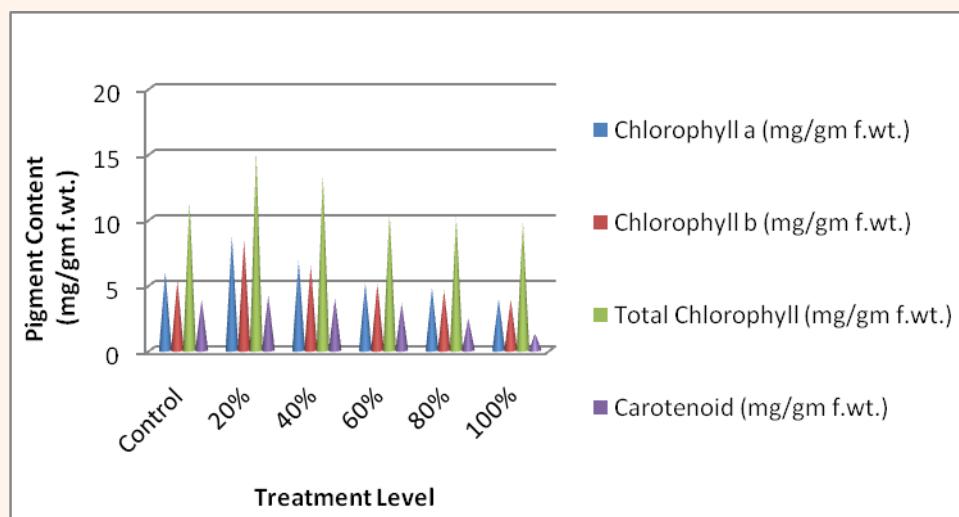
*Figures in parentheses represent % decrease over control.

**Figures in parentheses represent % increase over control.

FIGURE - 12



(A) Pigment Content (30 DAS)



(B) Pigment Content (60 DAS)

Graph showing Effect of different treatment levels of dyeing and printing effluent on Pigment content in *Glycine max L.* (Pot Experiment)

Observation and Results

**Table 13: Effect of dyeing and printing effluent on Yield parameters
of *Glycine max L.* after completion of life cycle (60 DAS)**

(Values are mean \pm Standard Deviation of 3 replicates)

S. No.	Treatment Level	Pods/Plant	Pods Weight/ Plant	Seeds/Pod	Seeds Weight/Pod
			(gm)		(gm)
1.	Control	8.66 \pm 0.009	4.13 \pm 0.019	2.33 \pm 0.029	3.6 \pm 0.105
2.	T ₁	11.66 \pm 0.015 (+34.64%)**	5.5 \pm 0.036 (+33.17%)**	3.33 \pm 0.058 (+42.91%)**	5.43 \pm 0.005 (+50.83%)**
3.	T ₂	9.66 \pm 0.025 (+11.54%)**	4.46 \pm 0.030 (+7.99%)**	2.66 \pm 0.019 (+14.16%)**	3.83 \pm 0.060 (+6.38%)**
4.	T ₃	7.33 \pm 0.020 (-15.35%)*	3.66 \pm 0.025 (-11.38%)*	2 \pm 0.005 (-14.16%)*	2.98 \pm 0.034 (-17.22%)*
5.	T ₄	6.66 \pm 0.034 (-23.09%)*	3.36 \pm 0.017 (-18.64%)*	1.66 \pm 0.011 (-28.75%)*	1.99 \pm 0.054 (-44.72%)*
6.	T ₅	5.33 \pm 0.005 (-38.45%)*	3.06 \pm 0.032 (-25.90%)*	1.33 \pm 0.030 (-42.91%)*	1.71 \pm 0.009 (-52.5%)*

*Figures in parentheses represent % decrease over control.

**Figures in parentheses represent % increase over control.

Observation and Results

Table 14: Effect of dyeing and printing effluent on Biochemical content (mg g⁻¹ fresh weight) of *Glycine max* L. after completion of life cycle (60 DAS)

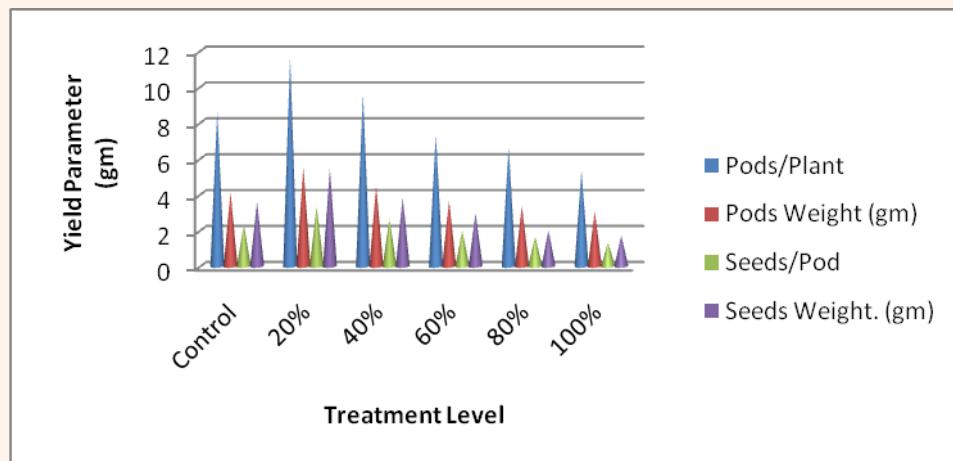
(Values are mean ± Standard Deviation of 3 replicates)

S. No.	Treatment Level	Protein (mg/gm f.wt.)	Free Amino Acid (mg/gm f.wt.)	Free Sugar (mg/gm f.wt.)
1	Control	13.21±0.028	7.26±0.037	11.18±0.070
2	T ₁	13.91±0.009 (+5.29%)**	10.45±0.034 (+43.93%)**	12.08±0.030 (+8.06%)**
3	T ₂	13.22±0.015 (+0.07%)**	7.14±0.0208 (-12.12%)*	11.58±0.020 (+3.56%)**
4	T ₃	10.79±0.005 (-18.31%)*	5.08±0.321 (-30.02%)*	11.04±0.017 (-1.21%)*
5	T ₄	9.95±0.010 (-24.67%)*	3.03±0.020 (-58.26%)*	10.86±0.026 (-2.83%)*
6	T ₅	7.22±0.023 (-45.34%)*	2.52±0.005 (-65.28%)*	10.40±0.017 (-6.99%)*

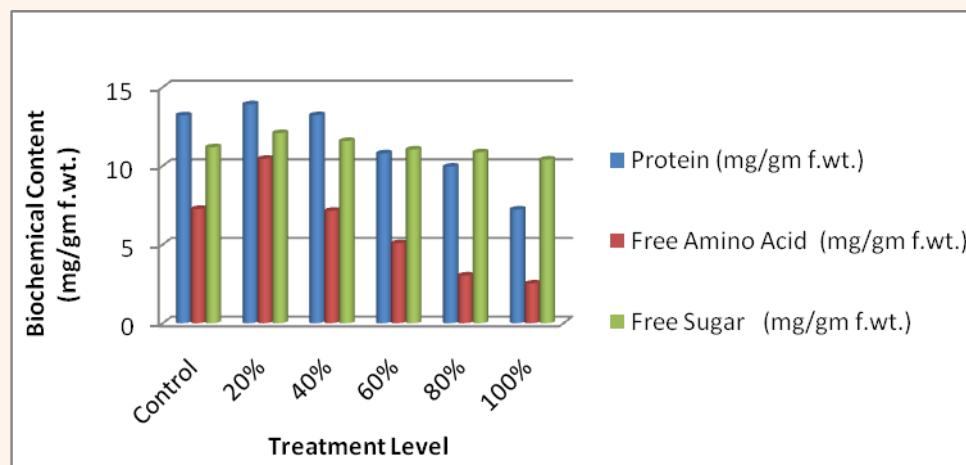
*Figures in parentheses represent % decrease over control.

**Figures in parentheses represent % increase over control.

FIGURE - 13



(A) Yield parameter



(B) Biochemical content

Graph showing Effect of different treatment levels of dyeing and printing effluent on various growth parameters in *Glycine max L.* (Pot Experiment)

4.4.4 Effect on Nodulation and Leghaemoglobin content in *Glycine max* L.

4.4.4.1: Nodulation Study

The nodules/plant and fresh weight and dry weight of nodules showed increasing trend as treated with 20% and 40% concentrated effluent (Table 15, Figure 14). There was no significant difference from the control, at 20% and 40% effluent concentration, but at higher concentration a decreasing effect on nodules/plant and fresh and dry weight of nodules was observed. The percentage of reduction in nodules/plant was 5.77%, 17.31% and 42.29% at T₃, T₄ and T₅ treatment level respectively when compared with control (17.3). Maximum nodules/plant (25.3) was observed at T₁ treatment level i.e. 46.16% increased as compared with control (17.3). Similar trends were observed in fresh and dry weight of nodules. Maximum fresh weight and dry weight of nodules (3.83gm and 2.23gm) was observed at T₁ treatment level i.e. 19.68% and 45.75% higher than control (3.2gm and 1.52gm respectively). Minimum fresh weight and dry weight was found at highest concentration of effluent (T₅) which was 2.5gm and 1.13gm respectively. A percentage in reduction was 21.87% and 26.14% respectively when compared with control.

4.4.4.2: Leghaemoglobin content

In *Glycine max* L. it was observed that effect of highly diluted effluent (20%) on leghaemoglobin content showed stimulatory effect rather than inhibitory effect (Table 16, Figure 14). The leghaemoglobin content at T₁ treatment level was 0.38 mg/gm at 590 A and 0.34 mg/gm at 556 A and At T₂ (40%) treatment level leghaemoglobin was 0.36 mg/gm at 590 A and 0.33 mg/gm at 556 A followed by control 0.35 mg/gm at 590 A and 0.32 mg/gm at 556 A. Percentage enhancement was found 10% at 590A and 5.47% at 556A in treatment level T₁ (20%) and 2.85% at 590A and 1.82% at 556A in T₂ (40%) over control, while highly reducing value

Observation and Results

of leghaemoglobin content was found in treatment level T_5 (0.257 mg/gm at 590A and 0.246 mg/gm at 556A) followed by gradual decreasing values in increasing concentration of effluent on treatment level T_3 (60%) 0.289 mg/gm at 590A and 0.277 mg/gm at 556A and T_4 (80%) 0.273 mg/gm at 590A and 0.266 mg/gm at 556A. Reduction in percentage value was found 20% at 590A and 15.80% at 556A in T_3 while 22% at 590A and 19.14% at 556A in T_4 treatment level in comparison to control.

Observation and Results

**Table 15: Effect of dyeing and printing effluent on Nodulation in *Glycine max* L.
after completion of life cycle (60 DAS)**

(Values are mean \pm Standard Deviation of 3 replicates)

S. No.	Treatment level	Nodules/Plant	Nodules fresh weight	Nodules dry weight
			(g/plant)	(g/plant)
1	Control	17.3 \pm 0.035	3.2 \pm 0.015	1.53 \pm 0.078
2	T ₁	25.3 \pm 0.029 (+46.16%)**	3.83 \pm 0.028 (+19.68%)**	2.23 \pm 0.012 (+45.75%)**
3	T ₂	19.3 \pm 0.025 (+11.54%)**	3.46 \pm 0.035 (+8.12%)**	1.51 \pm 0.038 (+1.30%)*
4	T ₃	16.3 \pm 0.009 (-5.77%)*	3.13 \pm 0.015 (-2.18%)*	1.46 \pm 0.009 (-4.57%)*
5	T ₄	14.3 \pm 0.011 (-17.31%)*	2.9 \pm 0.026 (-9.37%)*	1.33 \pm 0.011 (-13.07%)*
6	T ₅	10.3 \pm 0.010 (-42.29%)*	2.5 \pm 0.029 (-21.87%)*	1.13 \pm 0.099 (-26.14%)*

*Figures in parentheses represent % decrease over control.

**Figures in parentheses represent % increase over control.

Observation and Results

**Table 16: Effect on Leghaemoglobin content (mg g⁻¹ fresh nodule)
of *Glycine max* L. treated with dyeing and printing effluent**

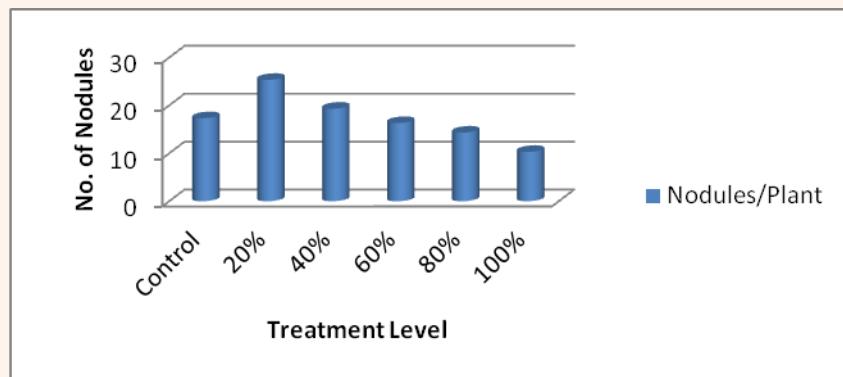
(Values are mean ± Standard Deviation of 3 replicates)

Treatment level	Leghaemoglobin (mg/gm)	
	<i>Glycine max</i> L.	
	590 nm.	556 nm.
Control	0.35±0.010	0.329±0.097
T ₁	0.385±0.026 (+10%) **	0.347±0.038 (+5.47%) **
T ₂	0.36±0.055 (+2.85%) **	0.335±0.022 (+1.82%) **
T ₃	0.289±0.015 (-20%) *	0.277±0.019 (-15.80%) *
T ₄	0.273±0.099 (-22%) *	0.266±0.038 (-19.14%) *
T ₅	0.257±0.038 (-26.57%) *	0.246±0.015 (-25.22%) *

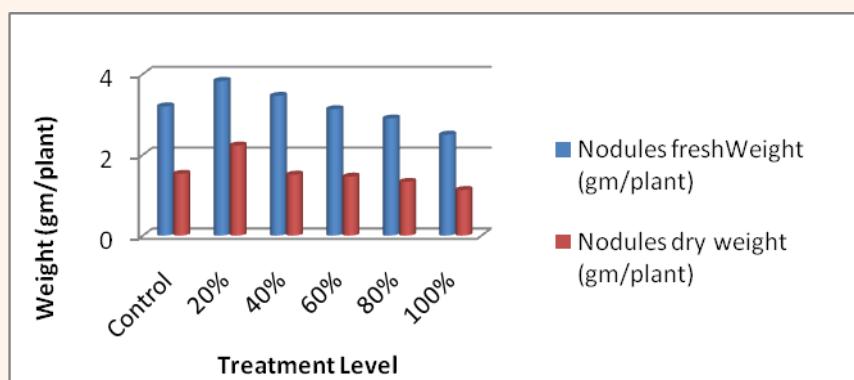
*Figures in parentheses represent % decrease over control

**Figures in parentheses represent % increase over control.

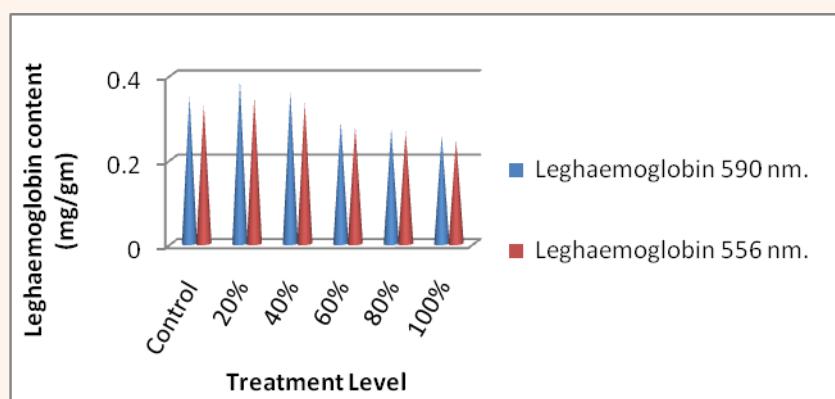
FIGURE - 14



(A) Nodules/Plant



(B) Nodules Weight (gm/plant)



(C) Leghaemoglobin content

Graph showing Effect of different treatment levels of dyeing and printing effluent on nodulation parameters in *Glycine max L.* (Pot Experiment)

4.5 Effect of dyeing and printing effluent on germination and seedling growth of *Medicago sativa L.*: (Petri plate Method)

The results of present research work reveals that higher concentrations of dyeing effluent adversely influence the seed germination and seedling growth of experimental plants. Germination study on seeds of the experimental plant i.e. *Medicago sativa L.* var. T9 has been done on 14th day of sowing.

Germination and seedling growth in Medicago sativa L.

The effects of effluent on germination and seedling growth of *Medicago sativa L.* are shown in Table 17 and Figure 15. The highest value of seed germination in *Medicago sativa L.* was observed at T₁ (20%) treatment level i.e. 90%, which is higher than percentage germination at control (83%). The percentage germination was reducing with the increasing concentration of effluent. In the treatments with 40%, 60%, 80% and 100% germination percentage was 81%, 66%, 50% and 33% respectively. At T₅ treatment level (100%) minimum germination percentage was observed.

The highest value of vigour index in *Medicago sativa L.* was observed at T₁ (20%) treatment level i.e. 945, followed by control 838.3, while decreasing pattern in vigour index values were found at T₂ (40%), T₃ (60%) and T₄ (80%) levels i.e. 793, 504, 370 respectively. Lowest vigour index value was recorded at T₅ (100%) treatment level i.e. 174.9 which is 79.1% decrease in comparison to control. At T₁ treatment level vigour index of *Medicago sativa L.* was 12.7% higher than control (Table 17, Figure 15).

Shoot length of *Medicago sativa L.* treated with dyeing industrial effluent was observed after 14th day of seed sowing. The highest shoot length (5.1 cm) was obtained in 20% (T₁) treatment level which is 4.0% higher in comparison to control where seedling growth was slightly reduced (4.9 cm) than T₁ treatment level. The seedlings treated with 40%, 60% 80% and 100% concentrated solutions of dyeing effluent, a gradual decline (4.8cm, 4.1cm, 2.6cm and 2.4cm respectively) was observed in shoot length. The length of shoot was highly reduced at 100% (T₅) treatment level i.e. 2.4 cm which is 51.0% decrease in comparison to control (Table 17, Figure 15).

Observation and Results

Root length of *Medicago sativa* L., was found maximum (5.4cm) at 20% (T_1) treatment level and minimum (2.9cm) at 100% of effluent concentration which was 3.8% higher and 44.2% lower respectively as compared to control (5.2cm). Decreasing values of length of root were obtained at T_2 , T_3 and T_4 treatment level i.e. 5.0cm, 4.3cm and 3.8cm respectively (Table 17, Figure 15).

Fresh weight of plant *Medicago sativa* L. varied from 1.77 gm to 0.52 gm. The minimum value was observed at T_5 (100% raw effluent) treatment level i.e. 0.52 gm while maximum value of fresh weight was found at T_1 (20%) treatment level i.e. 1.77 gm which is 12.7% higher than fresh weight at control (1.57gm). At T_2 , T_3 and T_4 treatment levels fresh weight of plant *Medicago sativa* L. was 1.52 gm, 1.39 gm and 0.95 gm respectively which were 3.18%, 11.4%, 39.4% respectively decreased in comparison to control (1.57 gm). Dry weight of plant *Medicago sativa* L. varied from 0.22 gm to 0.57 gm when they were treated with 20% to 100% dyeing effluent. Maximum value of dry weight (0.57gm) was observed at T_1 treatment level (20%) which is 11.7% increase in comparison to control 0.51 gm. The minimum dry weight (0.22 gm) of plant was recorded at T_5 (100%) treatment level which is 56.8% decreased in comparison to control (Table 17, Figure 16).

The pigment content in *Medicago sativa* L. were negatively affected when seedlings treated with different concentrations of dyeing and printing effluent (Table 17, Figure 16). The minimum value of Chlorophyll 'a' was found in plants grown in T_5 (100% raw effluent) treatment level i.e. 1.01 mg/gm fresh weight which was 66.6 % reduced in comparison to control where Chlorophyll content 'a' was 3.02 mg/gm fresh weight. Increased level of Chlorophyll 'a' was recorded in plants treated with T_1 treatment level i.e. 3.21 mg/gm fresh weight (6.2% higher than control). At T_2 , T_3 and T_4 treatment levels the value of Chlorophyll a was 2.58 mg/gm fresh weight, 2.03 mg/gm fresh weight and 1.92 mg/gm fresh weight respectively. Similarly the value of Chlorophyll 'b' was observed highest in T_1 (20%) treatment level 3.01 mg/ gm fresh weight which is 1.6% higher than control (2.96 mg/gm fresh weight). The decline in values of chlorophyll b content was recorded at

Observation and Results

Table 17: Effect of dyeing and printing effluent on germination and seedling growth of *Medicago sativa* L.: (Petri plate Method)

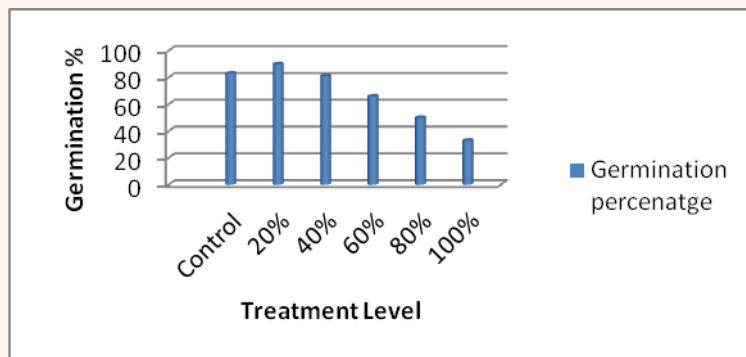
(Values are mean \pm Standard Deviation of 3 replicates)

S. No.	Treatment Level	Germination Percentage	Vigour Index	Seedling growth (14 ^t days)		Weight		Chlorophyll content (mg/gm tissue)	
				Shoot Length(cm)	Root Length(cm)	Fresh Weight (gm)	Dry Weight (gm)	Chlorophyl a (mg/gm)	Chlorophyl b (mg/gm)
1.	Control	83	838.3 \pm 0.011	4.9 \pm 0.022	5.2 \pm 0.021	1.57 \pm 0.021	0.51 \pm 0.005	3.02 \pm 0.021	2.96 \pm 0.001
2.	T ₁	90	945 \pm 0.021 (+12.7%)**	5.1 \pm 0.010 (+4.0%)**	5.4 \pm 0.010 (+3.8%)**	1.77 \pm 0.020 (+12.7%)**	0.57 \pm 0.010 (+11.7%)**	3.21 \pm 0.021 (+6.2%)**	3.01 \pm 0.011 (+1.6%)**
3.	T ₂	81	793 \pm 1.001 (-5.3%)*	4.8 \pm 0.002 (-2.04%)*	5.0 \pm 0.016 (-3.84%)*	1.52 \pm 0.026 (-3.18%)*	0.49 \pm 0.011 (-3.92%)*	2.58 \pm 0.015 (-14.5%)*	2.80 \pm 0.015 (-14.5%)*
4.	T ₃	66	504 \pm 0.020 (-33.8%)*	4.1 \pm 0.015 (-19.2%)*	4.3 \pm 0.011 (-17.3%)**	1.39 \pm 0.011 (-11.4%)*	0.42 \pm 0.015 (-17.6%)*	2.03 \pm 0.024 (-32.7%)*	2.56 \pm 0.014 (-13.5%)*
5.	T ₄	50	370 \pm 0.017 (-55.8%)*	3.6 \pm 0.005 (-26.5%)*	3.8 \pm 0.010 (-26.9%)*	0.95 \pm 0.021 (-39.4%)*	0.35 \pm 0.010 (-31.3%)*	1.92 \pm 0.017 (-36.4%)*	1.80 \pm 0.012 (-39.1%)*
6.	T ₅	33	174.9 \pm 0.010 (-79.1%)*	2.4 \pm 0.011 (-51.0%)*	2.9 \pm 0.011 (-44.2%)*	0.52 \pm 0.001 (-66.8%)*	0.22 \pm 0.010 (-56.8%)*	1.01 \pm 0.011 (-66.5%)*	1.05 \pm 0.011 (-64.5%)*

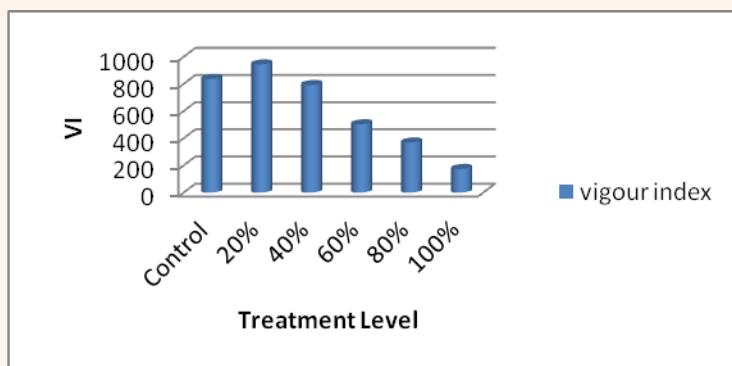
*Figures in parentheses represent % decrease over control.

**Figures in parentheses represent % increase over control

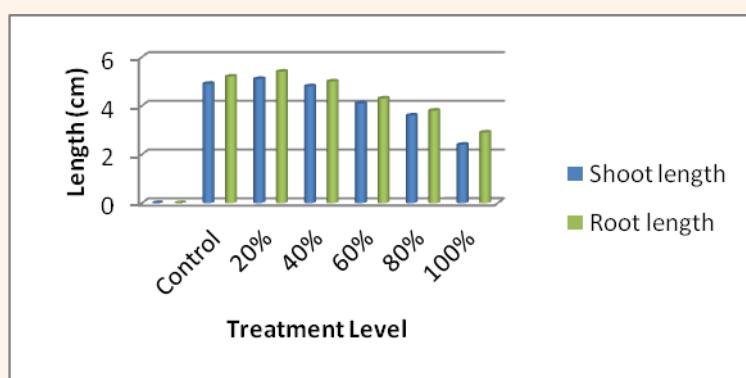
FIGURE - 15



(A) Germination Percentage



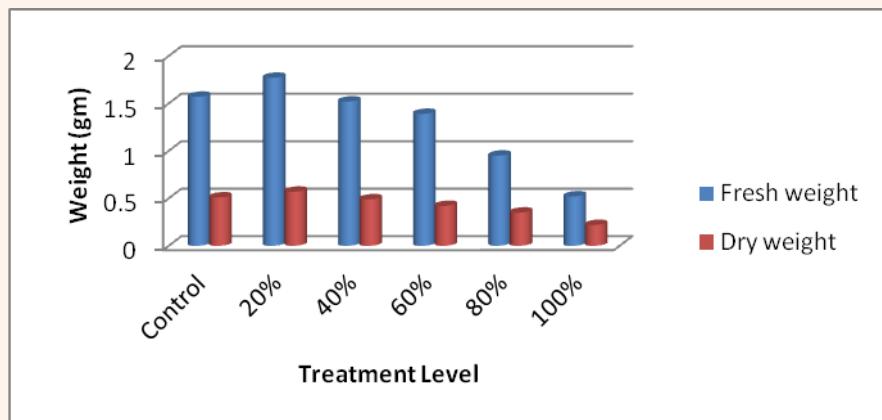
(B) Vigour Index



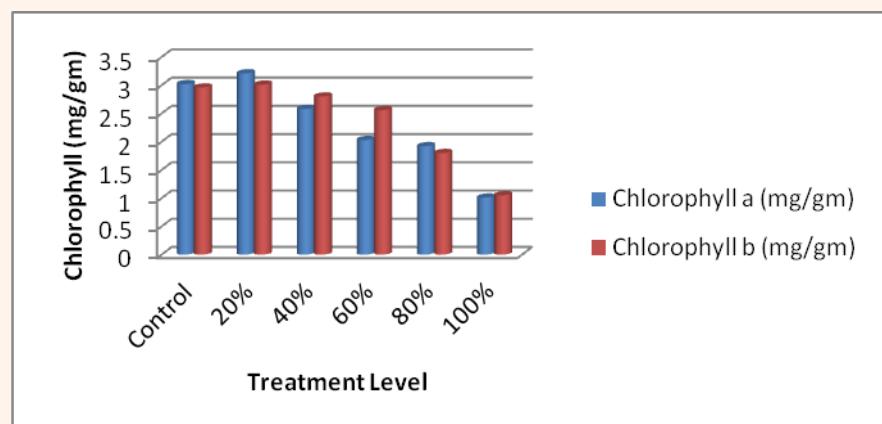
(C) Shoot length and Root length

Graph showing Effect of different treatment levels of dyeing and printing effluent on various growth parameters in *Medicago sativa L.* (Petri plate Method)

FIGURE - 16



(D) Fresh and Dry weight



(E) Chlorophyll Content

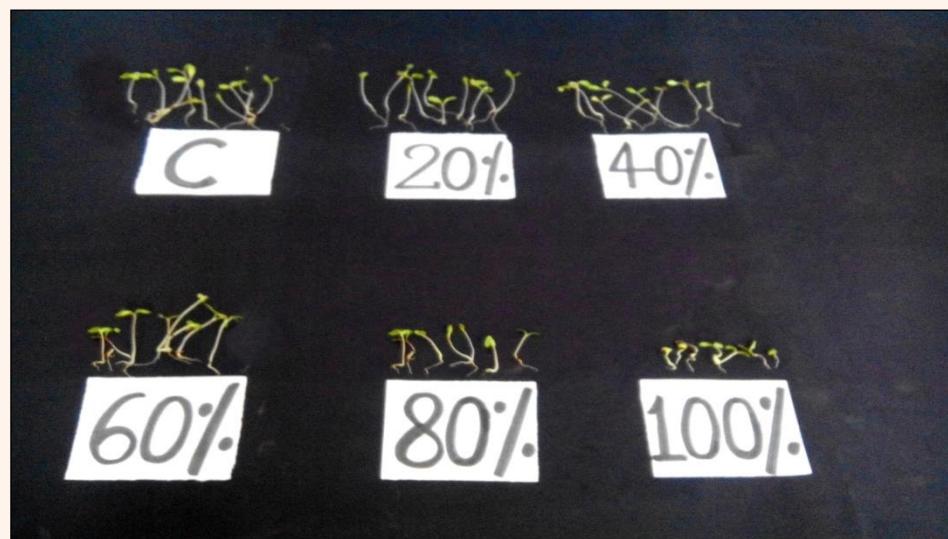
Graph showing Effect of different treatment levels of dyeing and printing effluent on various growth parameters in *Medicago sativa L.* (Petri plate Method)

PLATE - 11

Control 20% 40% 60% 80% 100%



(A) Percentage Germination



(B) Percentage Germination

**Showing Percentage Germination of *Medicago sativa* L. in
Different Treatment Levels of Dyeing and Printing Effluent
(Petri-Plate Method)**

T_2 (2.80 mg/gm fresh weight), T_3 (2.56 mg/gm fresh weight) and T_4 (1.80 mg/gm fresh weight) which were 14.5%, 13.5% and 39.1% decreased as compared with control. The lowest value of Chlorophyll 'b' was observed at T_5 level i.e. 1.05 mg/gm fresh weight which is 64.5% reduced in comparison to control.

4.6: Evaluation of various growth and biochemical parameters of *Medicago sativa* L. treated with different concentration of dying and printing effluent: (Pot Experiment)

Figure 17, 18, 19, 20, 21, 22 shows the relative degree of enhancement or inhibition on seedling growth (Shoot and Root growth, fresh and dry weight of shoot and root, vigour index), productivity, pigment content, biochemical parameters and nodulation. The effluent has significant effect on seed germination in early stage which has gradually decreased with increasing concentration of effluent. Higher concentration (60%, 80% and 100%) of effluent had inhibitory effect than that of lower concentration (20% and 40%). Various growth parameters were observed twice, which was on 30 day of sowing and after the completion of life cycle of the experimental crop i.e. *Medicago sativa* L. The results are presented in Table 18.

4.6.1 Effect on Growth and Pigment content of *Medicago sativa* L. after 30 day of sowing

(A) Shoot length

A gradual decline in shoot length of *Medicago sativa* L. was observed with increasing concentration of dyeing and printing effluent (Table 18 and Figure 17). At 30 DAS, the average highest shoot length was recorded in T_1 (20%) treatment level (24.53cm), followed by T_2 (40%) treatment level (20.76cm) and control (19.56cm). At T_1 and T_2 treatment level shoot length was increased 20.40% and 6.13% respectively as compare to control. Higher concentration of effluent had maximum inhibitory effect i.e. T_5 (100%) treatment level, where shoot length was 15.06cm which is 23.00% reduced in comparison to control.

(B) Root length

The effect of highly diluted effluent on root length in *Medicago sativa* L. shows stimulatory effect rather than inhibitory effect. At T₁ (20%) treatment level root length was 18.6cm followed by control 15.73cm, Percentage enhancement was found 18.24% in treatment level T₁ (20%) over control. While highly reducing value of root length was found in T₅ (100%) treatment level (9.83cm) followed by gradual decreasing values in increasing concentration of effluent at treatment level T₂ (40%), T₃ (60%) and T₄ (80%) i.e. 15.43cm, 15.2cm and 12.73cm respectively. Reduction in percentage value was found 1.90%, 3.36%, 19.07% and 37.50% in treatment level T₂-T₅ respectively in comparison to control (Table 18 and Figure 17).

(C) Fresh Weight of Shoot

Table 18 and Figure 18 illustrate the relative degree of enhancement or inhibition by effluent on fresh weight and dry weight of shoots and roots of *Medicago sativa* L. In case of fresh weight of shoots in *Medicago sativa* L., minimum value was observed on 30 days at treatment level T₅ (100% raw effluent) i.e. 9.02 gm/plant which was 31.87% decreased in comparison to control 13.24gm/plant while maximum value of fresh weight of shoot was observed at T₁ (20%) and T₂ (40%) treatment level i.e. 16.2 gm/plant and 15.22 gm/plant which was 22.35% and 14.95% respectively increased when compared with control (13.24 gm/plant).

(D) Fresh Weight of Root

Maximum fresh weight of plant roots treated with dyeing and printing effluent was found in treatment level T₁ (20%) and T₂ (40%) (9.43 gm/plant and 8.08 gm/plant respectively) which were 30.06% and 11.44% increased as compared with control (7.25 gm/plant). The fresh weight of root was decreased with the increasing concentration of effluent where fresh weight of root was 6.13 gm/plant, 4.81 gm/plant and 4.09 gm/plant at T₃, T₄ and T₅ treatment level respectively.

Observation and Results

Maximum reduction in fresh weight of root was at T₅ treatment level where fresh weight was decreased 43.58% as compared with control (Table 18 and Figure 18).

(E) Dry Weight of Shoot

Dry weight of shoot was ranged between 3.83 gm/plant to 2.11 gm/plant. Observations showed maximum values at 20% (T₁) effluent concentration i.e. 3.83 gm/plant which are 26.40% increased as compared with control 3.03 gm/plant. The dry weight of shoot inhibit when plants treated with higher concentration of effluent. Reduction in percentage increased from T₃ to T₅ i.e. 4.62%, 14.19% and 30.36% respectively (Table 18 and Figure 18).

(F) Dry Weight of Roots

At 30 days after sowing Figure 18 and Table 18 illustrate the relative degree of enhancement or inhibition by effluent on dry weight of roots. Dry weight of roots was relatively enhanced by 20% (T₁) that showed maximum value of dry weight of root i.e. 2.19 gm/plant followed by T₂ treatment level i.e. 2.09 gm/plant. At T₁ treatment level dry weight of root was increased 19.67% over control. Maximum reduction in dry weight of root was recorded at T₅ treatment level (1.14 gm/plant) which is 37.70% reduced when compared with control. The relative percentage inhibition in dry weight of root was found 17.48% at 60% effluent concentration and 24.59% at 80% of effluent concentration (Table 18 and Figure 18).

(G) Vigour Index

The vigour index varied from 3986.13 to 1869.75 between control to T₅ treatment level. The vigour index was decrease with the increasing concentration of effluent. The minimum value of vigour index was found at T₅ (100%) treatment level i.e. 1869.75 which was 44.36% decreased as compared with control 3360.90. At T₁ (20%) and T₂ (40%) treatment level vigour index value of *Medicago sativa* L. plant was higher than control. The percentage increase was 18.60% and 2.80% over control at T₁ and T₂ treatment level respectively (Table 18, Figure 19).

Observation and Results

(H) Pigment content

The chlorophyll and carotenoid content in the experimental plant was studied and results are presented in Table 20 and Figure 20. Increase level of pigment content was recorded when effluent was diluted 80% with the normal water i.e. T₁ treatment level. The chlorophyll ‘a’ and chlorophyll ‘b’ was found maximum at T₁ treatment level i.e. 4.36 mg/gm fresh weight and 4.26 mg/gm fresh weight respectively which were 4.05% and 32.71% higher than control 4.19 mg/gm fresh weight and 3.21 mg/gm fresh weight respectively. A gradual decline was observed with the higher concentration of effluent from T₃ to T₅ treatment level. The maximum reduction in chlorophyll ‘a’ and chlorophyll ‘b’ was 47.97% and 35.51% respectively at T₅ treatment level when compared with control.

It was observed that effect of highly diluted effluent on total chlorophyll content in plant shows stimulatory effect rather than inhibitory at T₁ (9.40 mg/gm fresh weight) treatment level followed by control 8.37 mg/gm fresh weight and percentage increase was found 12.30% over control. The reduction in percentage of total chlorophyll content was found in treatment level T₃ (7.02 mg/gm fresh weight) 16.12% followed by gradual decreasing values in increasing concentration of effluent on treatment level T₄ (6.46 mg/gm fresh weight) and T₅ (5.85mg/gm fresh weight) which were reduced 22.81% and 30.10% respectively as compared with control. The values of carotenoids varied from 3.19 mg/gm fresh weight to 2.33 mg/gm fresh weight when treated with various concentrations of effluent. Maximum carotenoid content was recorded at T₁ treatment level (3.19 mg/gm fresh weight) that was 8.13% enhance over control (2.95 mg/gm fresh weight). The minimum value of carotenoid content was at T₅ treatment level (2.33 mg/gm fresh weight) which was 21.01% decreased as compared with control.

4.6.2 Effect on various Growth and yield of *Medicago sativa* L. after completion of life cycle (60 DAS)

(A) Shoot length

The result showed (Table 19 and Figure 17) that plant growth of *Medicago sativa* L. was influenced by different concentrations of dying and printing effluent. At 60 DAS, the average highest shoot length was recorded in T₁ (20%) treatment level (30.91 cm), followed by T₂ (40%) treatment level (27.3 cm) and control (25.76 cm). At T₁ and T₂ treatment level shoot length was increased 19.99% and 5.98% respectively as compare to control. Higher concentration of effluent had maximum inhibitory effect i.e. at T₅ (100%) treatment level, where shoot length was 20.14cm which is 21.82% reduced in comparison to control. At T₃ and T₄ treatment level shoot length was reduced 9.47% and 10.95% when compared with control.

(B) Root length

At 60 days after sowing the relative degree of enhancement or inhibition by effluent on root length was observed (Table 19, Figure 17). Root length was relatively enhanced by T₁ (20%) that showed maximum value was obtained 21.42cm followed by T₂ (40%) treatment level 18.92cm while slight reduction in value of root length was obtained in control (16.96cm). The relative enhancement percentage in root length was found to be highest (10.36%) by 20% effluent concentration and (5.22%) by 40% treatment level over control. It was relatively inhibited in all other cases. Inhibition of root length were obtained from treatment level T₃ (60%), T₄ (80%) and T₅ (100%) i.e. 13.81cm, 12.94cm and 10.91cm respectively. The relative percentage inhibition in root length was found (9.35%) by 60% effluent concentration, (20.29%) by 80% and (26.53%) by 100% treatment level respectively to control.

(C) Fresh Weight of Shoots

In case of fresh weight of shoots, maximum reductive value was observed at 60 days after sowing in treatment level T_5 (100% raw effluent 8.54 g/plant (33.54%) while maximum value of fresh weight of shoots was found in T_1 (20%) treatment level 14.52 g/plant (13%) followed by control 12.85g/plant. Whereas gradual decline occurred in fresh weight values in treatment level T_2 (40%), T_3 (60%) and T_4 treatment level (80%) i.e. 12.21g/plant (4.98%), 11.04g (14.09%) and 9.80g (23.74%) respectively in comparison to control (Table 19 and Figure 18).

(D) Fresh Weight of Roots

A gradual decline in fresh weight of roots was observed with increasing concentration of effluent (Table 19 and Figure 18). Minimum value of fresh weight of roots in *Medicago sativa* L., was observed at treatment level T_5 (100% raw effluent) i.e. 5.02 gm/plant which was 45.13% decreased in comparison to control while maximum value of fresh weight of root was observed at T_1 (20%) and T_2 (40%) treatment level i.e. 11.85 gm/plant and 10.18 gm/plant which was 29.51% and 11.26% respectively increased when compared with control (9.15 gm/plant).

(E) Dry Weight of Shoot

The observation of dry weight of shoot was recorded in table 19. Observations showed maximum values at 20% (T_1) effluent concentration 3.26 gm/plant (11.56%) followed by T_2 (40%) effluent concentration 2.92 gm/plant and control 2.76 gm/plant. The values of dry weight of shoot decreased with the increasing treatment level. The maximum percentage reduction 41.32% (1.18 gm/plant) was observed at 100% treatment level (T_5 raw effluent) followed by treatment level T_3 (60%) and T_4 (80%) i.e. 19.07% (2.18 gm/plant) and 26.01% (1.76 gm/plant) in comparison to control (Table 19 and Figure 18).

(F) Dry Weight of Roots

Maximum value of dry weight of plant roots treated by dyeing and printing effluent was found in treatment level T₁ (20%) i.e. 1.62g per plant followed by T₂ (40%) treatment level 1.34g per plant and control 1.19 gm/plant. Percentage enhancement was found 32.33% in T₁ and 20.30% in T₂ over control. While significantly decreasing values were obtained with increasing effluent concentration starting with T₃ (60%) treatment level 1.05 gm/plant and T₄ (80%) treatment level 0.81 gm/plant and maximum reduction was obtained in T₅ (100%) raw effluent i.e. 0.74 gm/plant. Percentage reduction was found in treatment level T₃- T₅ i.e. 2.25%, 27.81% and 35.33% in comparison to control (Table 19, Figure 18).

(G) Vigour Index

The vigour index varied from 4513.90 to 2974.90 at control and T₁ to T₅ treatment level (Table 19 and Figure 19). The vigour index was decrease with the increasing concentration of effluent. The minimum value of vigour index was found at T₅ (100%) treatment level i.e. 2974.90 which was 23.91% decreased as compared with control 3909.51. At T₁ (20%) and T₂ (40%) treatment level vigour index value of *Medicago sativa* L. plant was higher than control. The percentage increase was 15.46% and 7.78% over control at T₁ and T₂ treatment level respectively.

(H) N, P, K Content

A gradual decline was observed in N, P, K Content with the increasing concentration of the effluent. In *Medicago sativa* L. Nitrogen content varied from 4.92% to 2.29%, Phosphorus content varied from 3.51% to 2.15% and Potassium content varied from 3.18% to 2.04%. The maximum Nitrogen content was observed at T₁ treatment level i.e. 4.92% followed by T₂ level i.e. 4.16%. The Nitrogen content was 41.37% and 19.54% enhanced at T₁ and T₂ treatment level respectively in comparison to control i.e. 3.48%. The inhibition of Nitrogen content was observed at T₃, T₄ and T₅ treatment level i.e. 3.19%, 2.55% and 2.29% respectively which were 8.33%, 26.72% and 34.19% respectively reduce as compared with

Observation and Results

control. The maximum Phosphorus content was observed at T₁ treatment level i.e. 3.51% followed by T₂ level i.e. 3.12%. The Phosphorus content was 18.58% and 5.40% enhanced at T₁ and T₂ treatment level respectively in comparison to control i.e. 2.96%. The inhibition of Phosphorus content was observed at T₃, T₄ and T₅ treatment level i.e. 2.81%, 2.49% and 2.15% respectively which were 5.06%, 15.87% and 27.36% respectively reduce as compared with control. Similarly the maximum Potassium content was observed at T₁ treatment level i.e. 3.18% followed by T₂ level i.e. 2.98%. The Potassium content was 11.57% and 4.56% enhanced at T₁ and T₂ treatment level respectively in comparison to control i.e. 2.85%. The inhibition of Potassium content was observed at T₃, T₄ and T₅ treatment level i.e. 2.71%, 2.42% and 2.04% respectively which were 4.91%, 15.08% and 28.42% respectively reduce as compared with control (Table 19 and Figure 19).

(I) Yield Parameters

The productivity of the treated plant (*Medicago sativa* L.) was also affected with the increased concentration of dying and printing effluent (Table 23 and Figure 21). The yield parameters viz. pod per plant, weight of pod per plant, no. of seeds per pod and weight of seed per pod were recorded after the completion of life cycle. The maximum pods/plant was observed at T₁ treatment level followed by T₂ and control. At T₁ and T₂ treatment level pods/plant was 10.33 and 8.31 which was 24% and 0.24% higher than control (8.33). The minimum pods/plant was observed at T₅ treatment level i.e. 5.66. The reduction in percentage was 32.05% when compared with control. A gradual decline in pods weight/plant of *Medicago sativa* L. was observed with increasing concentration of dying and printing effluent. The average highest pods weight/plant was recorded in T₁ (20%) treatment level (3.96 gm), followed by T₂ (40%) treatment level (3.83 gm) and control (3.73 gm). At T₁ and T₂ treatment level pods weight/plant was increased 6.16% and 2.68% respectively as compare to control. Higher concentration of effluent had maximum inhibitory effect i.e. T₅ (100%) treatment level, where pods weight/plant was 2.6 gm which is 30.29% reduced in comparison to control.

Observation and Results

The seed/pod was higher at 20% (3.66) and 40% (3.0) effluent than that of control (2.66). Increase in effluent concentration from 60% onwards reduced the no. of seeds per pod. The minimum no. of seeds/pod (1.66) was found at T₅ treatment level that was 37.59% reduced when compared with control. Maximum seed weight/pod was found in plant treated with 20% concentrated effluent (T₁) and 40% concentrated effluent (T₂). The seed weight/pod in *Medicago sativa* L. was increased 5.74% (3.13gm) at T₁ level and 3.37% (3.06gm) as compared with control (2.96 gm). The seed weight/pod was decreased with the increasing concentration of effluent where seed weight/pod was 2.86 gm, 2.66 gm and 2.26 gm at T₃, T₄ and T₅ treatment level. Maximum reduction in seed weight/pod was at T₅ treatment level where seed weight was decreased 23.64% as compared with control.

4.6.3 Effect on biochemical parameters of *Medicago sativa* L.

4.6.3.1: Pigment content

The pigment content was also recorded after the completion of life cycle of the treated *Medicago sativa* L. plant. Results showed (Table 21, Figure 21) that the value of chlorophyll ‘a’, chlorophyll ‘b’ and carotenoid content was higher when plants treated with 20% and 40% concentrated effluent. At T₁ chlorophyll ‘a’ and chlorophyll ‘b’ was 6.08 mg/gm fresh weight and 11.20 mg/gm fresh weight followed by T₂ (5.52 mg/gm fresh weight and 9.41 mg/gm fresh weight respectively) and control (5.4 mg/gm fresh weight and 9.2 mg/gm fresh weight respectively). A decreasing pattern was observed in the values of all the pigment content with the increasing concentration of effluent from T₃ to T₅ (60% to 100%). The maximum reduction in chlorophyll ‘a’ and ‘b’ was found at T₅ treatment level (4.44 mg/gm fresh weight and 7.88 mg/gm fresh weight respectively) which were 17.77% and 14.34% reduced when compared with control. Total chlorophyll content in *Medicago sativa* L. was also adversely affected with the increasing concentration of effluent. The values of carotenoids varied from 3.63 mg/gm fresh weight to 1.6 mg/gm fresh weight when treated with various concentrations of effluent. Maximum carotenoid content was recorded at T₁ treatment level (3.63 mg/gm fresh weight) that was 19.01% enhance over control (3.05 mg/gm fresh

Observation and Results

weight). The minimum value of carotenoid content was at T₅ treatment level (1.6 mg/gm fresh weight) which was 47.54% decreased as compared with control.

4.6.3.2: Protein

The protein content in *Medicago sativa* L. was negatively affected when treated with higher concentration of dyeing and printing effluent (Table 22 and Figure 21). As effluent concentration increased from T₃ (60%) to T₅ (100%) the protein content was adversely affected. The maximum reduction was found at T₅ (100% effluent) treatment level i.e. 12.23 mg/gm fresh weight which decreased 6.42% as compared with control. Increased protein content was recorded at T₁ treatment level i.e. 13.21 mg/gm fresh weight which enhance 1.07% over control (13.07 mg/gm fresh weight). The value of protein was approximately similar in plants treated with 40% effluent (13.09 mg/gm fresh weight) and control.

4.6.3.3: Free Amino acid

Medicago sativa L. plants when treated with various concentration of dyeing and printing effluent the free amino acid ranged between 7.15 mg/gm fresh weight to 5.91 mg/gm fresh weight. Highest amount (7.15 mg/gm fresh weight) of free amino acid was observed at T₁ treatment level which was 7.51% increased over control. A gradual decline in free amino acid content was observed with the increasing concentration of effluent. At T₃, T₄ and T₅ the free amino acid was 6.44 mg/gm fresh weight, 6.18 mg/gm fresh weight, 5.91 mg/gm fresh weight respectively which were 3.15%, 7.06% and 11.12% reduced when compared with control (6.65 mg/gm fresh weight). The difference in the value of free amino acid at T₂ (6.69 mg/gm fresh weight) and control is negligible (Table 22 and Figure 21).

4.6.3.4: Free Sugar

Free sugar in treated plant was also adversely affected with the increasing concentration of effluent. The maximum (11.30 mg/gm fresh weight) free sugar content was recorded at T₁ treatment level which was 3.29% higher when compared with control 10.94 mg/gm fresh weight. At T₂ treatment level also free sugar content was 1.18% increased over the control. The minimum (9.66 mg/gm fresh weight) free sugar content was recorded at T₅ treatment level which was 11.70% decreased when compared with control (Table 22 and Figure 21).

Observation and Results

**Table 18: Effect of dyeing and printing effluent on seedling growth of
Medicago sativa L. after 30 day of sowing**

(Values are mean \pm Standard Deviation of 3 replicates)

S. No.	Treatment Level	Shoot Length (cm)	Root Length (cm)	Shoot Fresh Weight (gm/plant)	Root Fresh Weight (gm/plant)	Shoot Dry Weight (gm/plant)	Root Dry Weight (gm/plant)	Vigour Index
1.	Control	19.56 \pm 0.059	15.73 \pm 0.033	13.24 \pm 0.010	7.25 \pm 0.021	3.03 \pm 0.058	1.83 \pm 0.050	3360.90 \pm 4.421
2.	T ₁	24.53 \pm 0.036 (+20.40%)**	18.6 \pm 0.027 (+18.24%)**	16.2 \pm 0.035 (+22.35%)**	9.43 \pm 0.015 (+30.06%)**	3.83 \pm 0.019 (+26.40%)**	2.19 \pm 0.020 (+19.67%)**	3986.13 \pm 3.541 (+18.60%)**
3.	T ₂	20.76 \pm 0.351 (+6.13%)**	15.43 \pm 0.031 (-1.90%)*	15.22 \pm 0.009 (+14.95%)**	8.08 \pm 0.025 (+11.44%)**	3.01 \pm 0.051 (-0.66%)*	2.09 \pm 0.011 (+14.20%)**	3455.24 \pm 3.891 (+2.80%)**
4.	T ₃	18.6 \pm 0.251 (-4.90%)*	15.2 \pm 0.040 (-3.36%)*	12.73 \pm 0.011 (-3.85%)*	6.13 \pm 0.070 (-15.44%)*	2.89 \pm 0.032 (-4.62%)*	1.51 \pm 0.011 (-17.48%)*	2707.24 \pm 3.960 (-19.44%)*
5.	T ₄	17.33 \pm 0.458 (-11.40%)*	12.73 \pm 0.011 (-19.07%)*	10.26 \pm 0.015 (-22.50%)*	4.81 \pm 0.050 (-33.65%)*	2.60 \pm 0.011 (-14.19%)*	1.38 \pm 0.057 (-24.59%)*	2119.23 \pm 1.481 (-36.94%)*
6.	T ₅	15.06 \pm 0.3 (-23.00%)*	9.83 \pm 0.020 (-37.50%)*	9.02 \pm 0.019 (-31.87%)*	4.09 \pm 0.011 (-43.58%)*	2.11 \pm 0.041 (-30.36%)*	1.14 \pm 0.017 (-37.70%)*	1869.75 \pm 2.937 (-44.36%)*

*Figures in parentheses represent % decrease over control.

**Figures in parentheses represent % increase over control.

Observation and Results

**Table 19: Effect of dyeing and printing effluent on seedling growth of *Medicago sativa* L.
after completion of life cycle (60 DAS)**

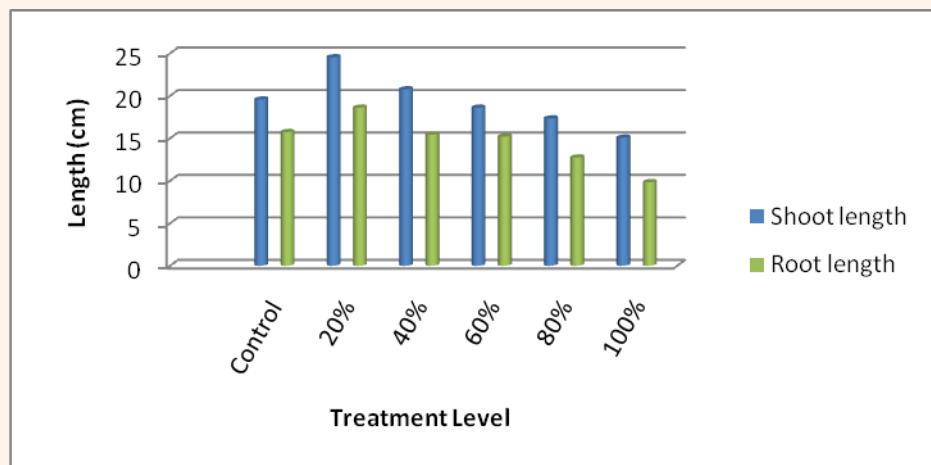
(Values are mean \pm Standard Deviation of 3 replicates)

S. No.	Treatment Level	Shoot Length (cm)	Root Length (cm)	Shoot Fresh Weight (gm/plant)	Root Fresh Weight (gm/plant)	Shoot Dry Weight (gm/plant)	Root Dry Weight (gm/plant)	Vigour Index	N, P, K %Content in Seedling		
									N%	P %	K %
1.	Control	25.76 \pm 0.047	16.96 \pm 0.037	12.85 \pm 0.160	9.15 \pm 0.041	2.76 \pm 0.057	1.19 \pm 0.208	3909.51 \pm 6.507	3.48%	2.96%	2.85%
2.	T ₁	30.91 \pm 0.188 (+19.99%)**	21.42 \pm 0.016 (+10.36%)**	14.52 \pm 0.018 (+12.99%)**	11.85 \pm 0.021 (+29.50%)**	3.26 \pm 0.251 (+18.11%)**	1.62 \pm 0.115 (+36.13%)**	4513.90 \pm 3.791 (+15.45%)**	4.92 \pm 0.081 (+41.37%)	3.51 \pm 0.012 (+18.58%)	3.18 \pm 0.091 (+11.57%)
3.	T ₂	27.3 \pm 0.081 (+5.97%)**	18.92 \pm 0.028 (+5.22%)**	12.21 \pm 0.029 (-4.98%)*	10.18 \pm 0.020 (+11.25%)**	2.92 \pm 0.2 (+5.79%)**	1.34 \pm 0.173 (+12.60%)**	4213.84 \pm 5.170 (+7.78%)**	4.16 \pm 0.009 (+19.54%)	3.12 \pm 0.010 (+5.40%)	2.98 \pm 0.004 (+4.56%)
4.	T ₃	23.32 \pm 0.012 (-9.47%)*	13.81 \pm 0.012 (-9.35%)**	11.04 \pm 0.121 (-14.08%)*	8.68 \pm 0.033 (-5.13%)*	2.18 \pm 0.099 (-21.01%)*	1.05 \pm 0.026 (-11.76%)*	3521.94 \pm 5.542 (-9.91%)*	3.19 \pm 0.190 (-8.33%)	2.81 \pm 0.049 (-5.06%)	2.71 \pm 0.051 (-4.91%)
5.	T ₄	22.94 \pm 0.009 (-10.94%)*	12.94 \pm 0.008 (-20.29%)*	9.80 \pm 0.004 (-23.73%)*	7.22 \pm 0.024 (-21.09%)*	1.76 \pm 0.030 (-36.23%)*	0.81 \pm 0.032 (-31.93%)*	3119.56 \pm 4.543 (-20.20%)*	2.55 \pm 0.185 (-26.72%)	2.49 \pm 0.015 (-15.87%)	2.42 \pm 0.121 (-15.08%)
6.	T ₅	20.14 \pm 0.044 (-21.81%)*	10.91 \pm 0.151 (-26.53%)*	8.54 \pm 0.049 (-33.54%)*	5.02 \pm 0.072 (-45.13%)*	1.18 \pm 0.230 (-57.24%)*	0.74 \pm 0.035 (-37.81%)*	2974.90 \pm 4.212 (-23.90%)*	2.29 \pm 0.016 (-34.19%)	2.15 \pm 0.001 (-27.36%)	2.04 \pm 0.081 (-28.42%)

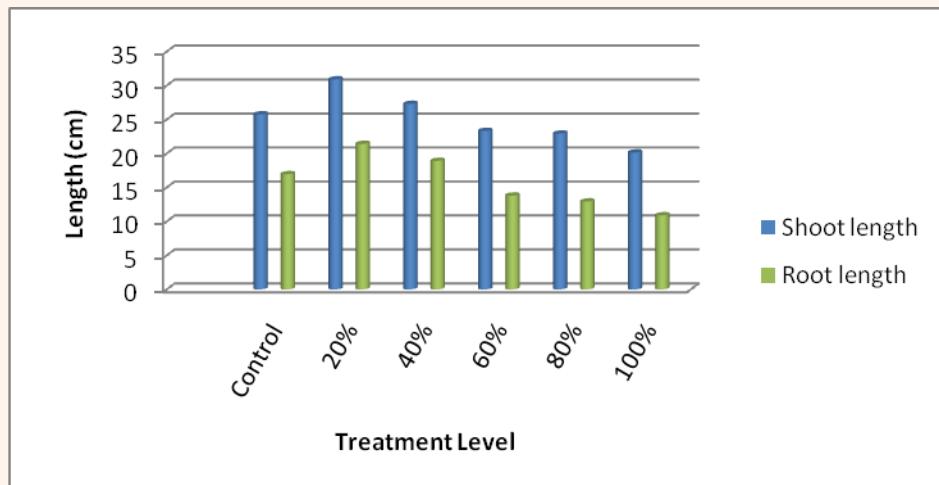
*Figures in parentheses represent % decrease over control.

**Figures in parentheses represent % increase over control.

FIGURE - 17



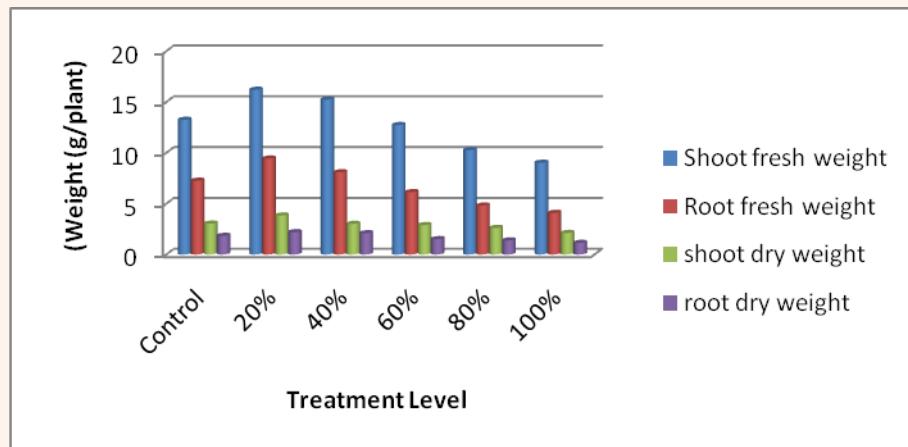
(A) Shoot and Root length (30 DAS)



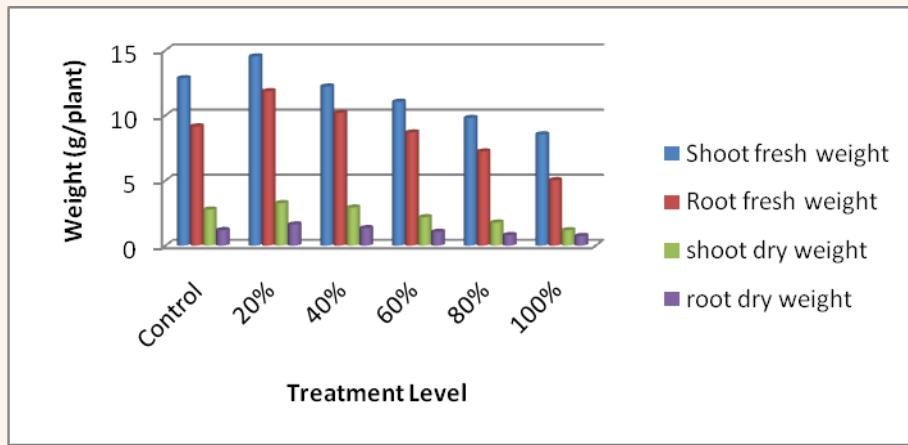
(B) Shoot and Root length (60 DAS)

Graph showing Effect of different treatment levels of dyeing and printing effluent on various growth parameters in *Medicago sativa* L. (Pot Experiment)

FIGURE - 18



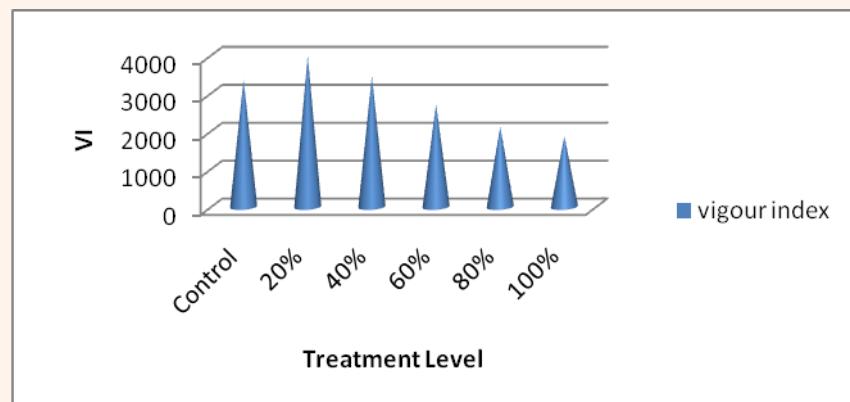
(A) Fresh and Dry Weight of Shoot and Root (30 DAS)



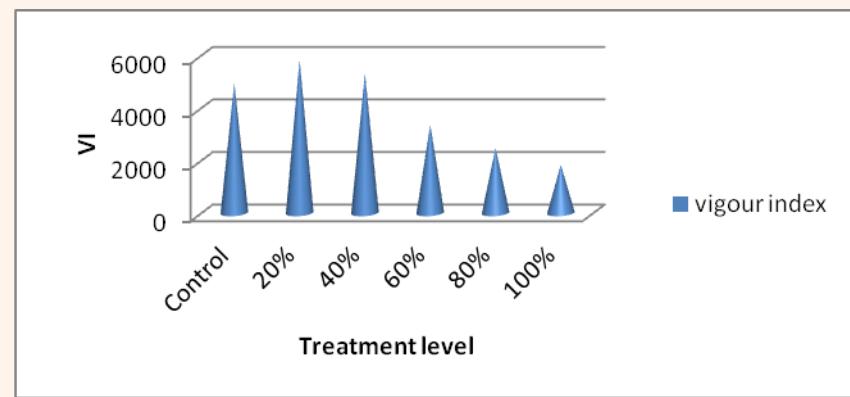
(B) Fresh and Dry Weight of Shoot and Root (60 DAS)

Graph showing Effect of different treatment levels of dyeing and printing effluent on various growth parameters in *Medicago sativa* L. (Pot Experiment)

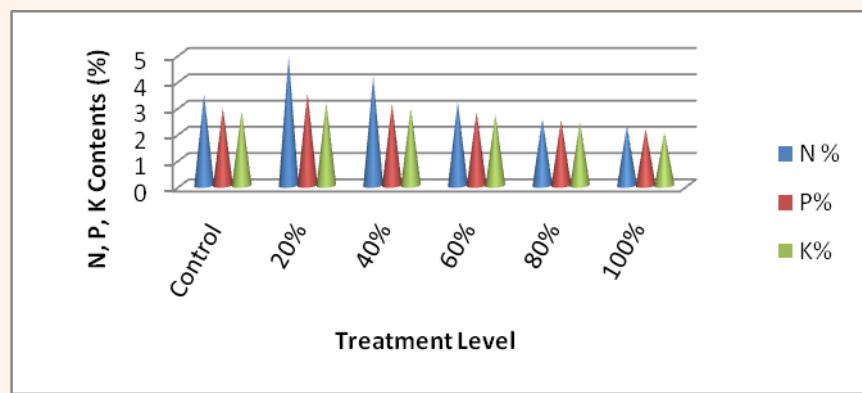
FIGURE – 19



(A) Vigour Index (30 DAS)



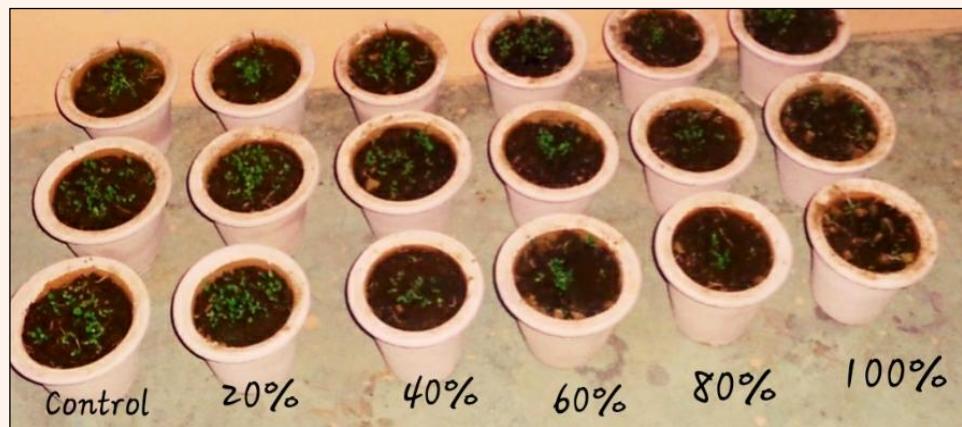
(B) Vigour Index (60 DAS)



(C) N, P, K Contents (60 DAS)

Graph showing Effect of different treatment levels of dyeing and printing effluent on various growth parameters in *Medicago sativa L.* (Pot Experiment)

PLATE - 12



(A) *Medicago sativa* L. Showing Growth in Pot (i)



(B) *Medicago sativa* L. Showing Growth in Pot (ii)



(C) *Medicago sativa* L. Showing Growth in Pot (iii)

Pot Experiment

Observation and Results

Table 20: Effect of dyeing and printing effluent on pigment content (mg g^{-1} fresh weight) of *Medicago sativa* L. after 30 day of sowing

(Values are mean \pm Standard Deviation of 3 replicates)

S. No	Treatment Level	Chlorophyll a (mg/gm f.wt.)	Chlorophyll b (mg/gm f.wt.)	Total Chlorophyll (mg/gm f.wt.)	Carotenoid (mg/gm f.wt.)
1.	Control	4.19\pm0.019	3.21\pm0.015	8.37\pm0.032	2.95\pm0.015
2.	T ₁	4.36\pm0.251 (+4.05%)**	4.26\pm0.026 (+32.71%)**	9.40\pm0.036 (+12.30%)**	3.19\pm0.017 (+8.13%)**
3.	T ₂	4.27\pm0.020 (+1.90%)**	3.65\pm0.030 (+13.70%)**	8.71\pm0.045 (+4.06%)**	3.08\pm0.029 (+4.40%)**
4.	T ₃	3.64\pm0.037 (-13.12%)*	3.18\pm0.045 (-0.93%)*	7.02\pm0.009 (-16.12%)*	2.90\pm0.005 (-1.69%)*
5.	T ₄	3.01\pm0.011 (-28.16%)*	2.71\pm0.035 (-15.57%)*	6.46\pm0.040 (-22.81%)*	2.84\pm0.041 (-3.72%)*
6.	T ₅	2.18\pm0.058 (-47.97%)*	2.07\pm0.020 (-35.51%)*	5.85\pm0.028 (-30.10%)*	2.33\pm0.020 (-21.01%)*

*Figures in parentheses represent % decrease over control.

**Figures in parentheses represent % increase over control.

Observation and Results

Table 21: Effect of dyeing and printing effluent on pigment content (mg g^{-1} fresh weight) of *Medicago sativa* L. after completion of life cycle (60 DAS)

(Values are mean \pm Standard Deviation of 3 replicates)

S. No	Treatment Level	Chlorophyll a (mg/gm tissue)	Chlorophyll b (mg/gm tissue)	Total Chlorophyll (mg/gm tissue)	Carotenoid (mg/gm tissue)
1.	Control	5.4 \pm 0.099	9.2 \pm 0.057	14.61 \pm 0.011	3.05 \pm 0.029
2.	T ₁	6.08 \pm 0.015 (+12.59%)**	11.20 \pm 0.010 (+21.73%)**	17.27 \pm 0.043 (+18.20%)**	3.63 \pm 0.009 (+19.01%)**
3.	T ₂	5.52 \pm 0.030 (+2.22%)**	9.41 \pm 0.020 (+2.28%)**	14.81 \pm 0.023 (+1.36%)**	3.13 \pm 0.015 (+2.62%)**
4.	T ₃	5.35 \pm 0.019 (-0.92%)*	9.04 \pm 1.192 (-1.73%)*	14.49 \pm 0.017 (-0.82%)*	2.59 \pm 0.055 (-15.08%)*
5.	T ₄	4.68 \pm 0.025 (-13.33%)*	8.68 \pm 0.026 (-5.65%)*	14.26 \pm 0.009 (-2.39%)*	1.92 \pm 0.010 (-37.04%)*
6.	T ₅	4.44 \pm 0.028 (-17.77%)*	7.88 \pm 0.015 (-14.34%)*	12.45 \pm 0.005 (-14.78%)*	1.6 \pm 0.020 (-47.54%)*

*Figures in parentheses represent % decrease over control.

**Figures in parentheses represent % increase over control.

Observation and Results

Table 22: Effect of dyeing and printing effluent on Biochemical contents (mg g^{-1} fresh weight) of *Medicago sativa* L. after completion of life cycle (60 DAS)

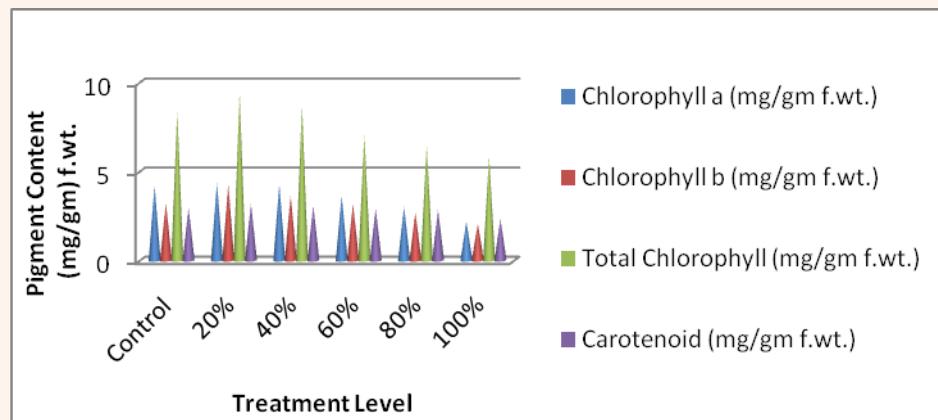
(Values are mean \pm Standard Deviation of 3 replicates)

S. No.	Treatment Level	Protein (mg/gm f.wt.)	Free Amino Acid (mg/gm f.wt.)	Free sugar (mg/gm f.wt.)
1	Control	13.07 \pm 0.023	6.65 \pm 0.030	10.94 \pm 0.015
2	T ₁	13.21 \pm 0.005 (+1.07%)**	7.15 \pm 0.017 (+7.51%)**	11.30 \pm 0.100 (+3.29%)**
3	T ₂	13.09 \pm 0.015 (+0.15%)**	6.69 \pm 0.035 (+0.60%)**	10.81 \pm 0.005 (-1.18%)*
4	T ₃	12.94 \pm 0.036 (-0.99%)*	6.44 \pm 0.051 (-3.15%)*	10.08 \pm 0.020 (-7.86%)*
5	T ₄	12.81 \pm 0.032 (-1.98%)*	6.18 \pm 0.029 (-7.06%)*	9.91 \pm 0.015 (-9.41%)*
6	T ₅	12.23 \pm 0.009 (-6.42%)*	5.91 \pm 0.005 (-11.12%)*	9.66 \pm 0.017 (-11.70%)*

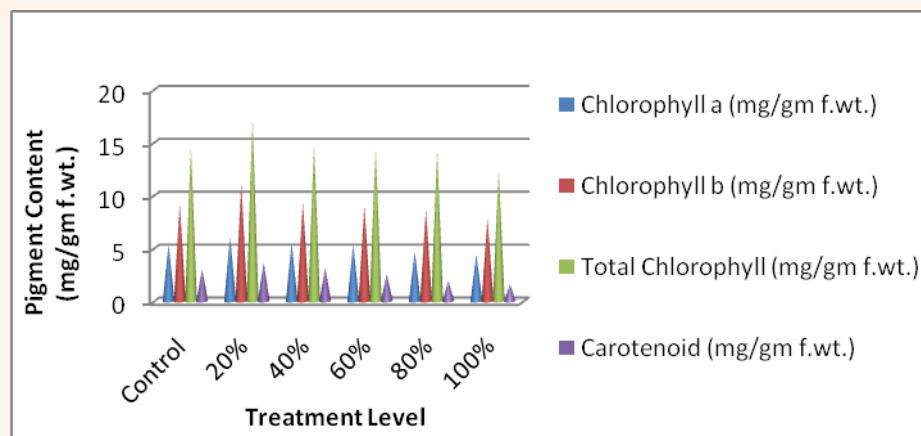
*Figures in parentheses represent % decrease over control.

**Figures in parentheses represent % increase over control.

FIGURE - 20



(A) Pigment Content (30 DAS)



(B) Pigment Content (60 DAS)

Graph showing Effect of different treatment levels of dyeing and printing effluent on Pigment content in *Medicago sativa* L. (Pot Experiment)

Observation and Results

Table 23: Effect of dyeing and printing effluent on Yield parameters of *Medicago sativa* L. after completion of life cycle (60 DAS)

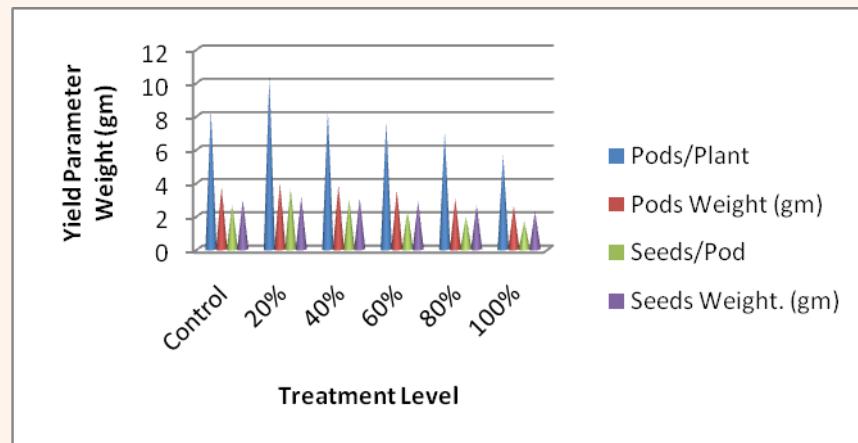
(Values are mean ± Standard Deviation of 3 replicates)

S. No.	Treatment Level	Pods/Plant	Pods Weight/ Plant	Seeds/Pod	Seeds Weight/Pod
			(gm)		(gm)
1.	Control	8.33±0.012	3.73±0.035	2.66±0.021	2.96±0.011
2.	T ₁	10.33±0.016 (+24%)**	3.96±0.019 (+6.16%)**	3.66±0.305 (+37.59%)**	3.13±0.015 (+5.74%)**
3.	T ₂	8.31±0.021 (-0.24%)*	3.83±0.025 (+2.68%)**	3±0.0199 (+12.78%)**	3.06±0.029 (+3.37%)**
4.	T ₃	7.66±0.001 (-8.04%)*	3.53±0.032 (-5.36%)*	2.33±0.011 (-12.40%)*	2.86±0.017 (-3.37%)*
5.	T ₄	7±0.011 (-15.96%)*	3.03±0.017 (-18.76%)*	2±0.015 (-24.81%)*	2.66±0.005 (-10.13%)*
6.	T ₅	5.66±0.007 (-32.05%)*	2.6±0.005 (-30.29%)*	1.66±0.011 (-37.59%)*	2.26±0.019 (-23.64%)*

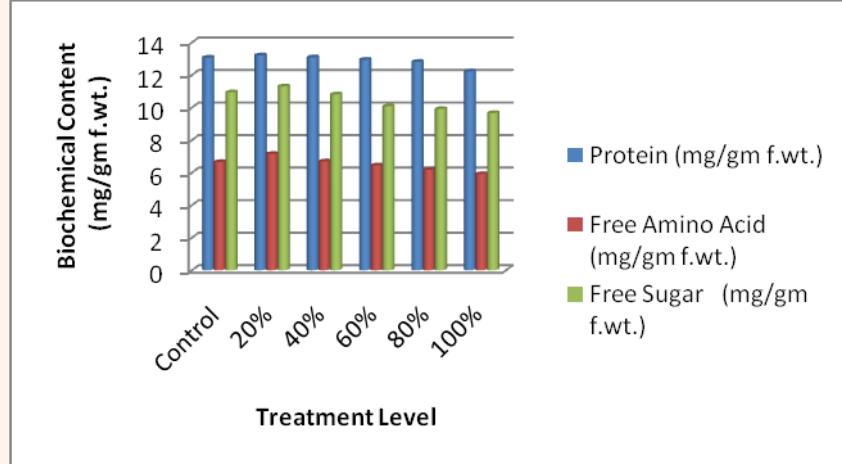
*Figures in parentheses represent % decrease over control.

**Figures in parentheses represent % increase over control.

FIGURE - 21



(A) Yield parameter



(B) Biochemical content

Graph showing Effect of different treatment levels of dyeing and printing effluent on various growth parameters in *Medicago sativa L.* (Pot Experiment)

4.6.4: Effect on Nodulation and Leghaemoglobin content in *Medicago sativa* L.

4.6.4.1: Nodulation study

The nodules/plant and fresh weight and dry weight of nodules showed increasing trend as treated with 20% and 40% concentrated effluent (Table 24 and Figure 22). There was no significant difference in values at control and 40% concentration of effluent (T_2), but at higher concentration a decreasing effect on nodules/plant and fresh and dry weight of nodules was observed. The percentage of reduction in nodules/plant was 12.77%, 19.15% and 38.31% at T_3 , T_4 and T_5 treatment level respectively when compared with control (15.66). Maximum nodules/plant (18.33) was observed at T_1 treatment level i.e. 17.04% increased as compared with control (15.66). Similar trends were observed in fresh and dry weight of nodules. Maximum fresh weight and dry weight of nodules (3.1gm/plant and 1.93gm/plant) was observed at T_1 treatment level i.e. 12.31% and 32.19% higher than control (2.76gm/plant and 1.46gm/plant respectively). Minimum fresh weight and dry weight was found at highest concentration of effluent (T_5) which was 1.86 gm/plant and 0.66 gm/plant respectively. A percentage in reduction was 32.60% and 54.79% respectively when compared with control.

4.6.4.2: Leghaemoglobin content

In *Medicago sativa* L. it was observed (Table 25 and Figure 22) that effect of highly diluted effluent (20%) on leghaemoglobin content showed stimulatory effect rather than inhibitory effect. The leghaemoglobin content at T_1 treatment level was 0.36 mg/gm fresh nodule at 590 A and 0.22 mg/gm fresh nodule at 556 A and At T_2 (40%) treatment level leghaemoglobin was 0.34 mg/gm fresh nodule at 590 A and 0.21 mg/gm fresh nodule at 556 A followed by control 0.29 mg/gm fresh nodule at 590 A and 0.20 mg/gm fresh nodule at 556 A. Percentage enhancement was found 24.39% at 590A and 12.25% at 556A in treatment level T_1 (20%) and 19.58% at 590A and 5.39% at 556A in T_2 (40%) over control, while highly reducing value of leghaemoglobin content was found in treatment level T_5 (0.22 mg/gm fresh nodule at 590A and 0.13 mg/gm

Observation and Results

Table 24: Effect of dyeing and printing effluent on Nodulation of *Medicago sativa* L. after completion of life cycle (60 DAS)

(Values are mean \pm Standard Deviation of 3 replicates)

S. No.	Treatment level	Nodules/Plant	Nodules freshWeight	Nodules dry weight
			(gm/plant)	(gm/plant)
1	Control	15.66 \pm 0.012	2.76 \pm 0.012	1.46 \pm 0.009
2	T ₁	18.33 \pm 0.051 (+17.04%)**	3.1 \pm 0.078 (+12.31%) **	1.93 \pm 0.020 (+32.19%)**
3	T ₂	16.66 \pm 0.011 (+6.38%)**	2.9 \pm 0.058 (+5.07%) **	1.36 \pm 0.016 (-6.84%) *
4	T ₃	13.66 \pm 0.028 (-12.77) *	2.43 \pm 0.013 (-11.95%) *	1.2 \pm 0.025 (-17.80%)*
5	T ₄	12.66 \pm 0.088 (-19.15%)*	2.26 \pm 0.011 (-18.11%) *	1 \pm 0.010 (-31.50%) *
6	T ₅	9.66 \pm 0.099 (-38.31%) *	1.86 \pm 0.017 (-32.60%) *	0.66 \pm 0.010 (-54.79%) *

*Figures in parentheses represent % decrease over control.

**Figures in parentheses represent % increase over control.

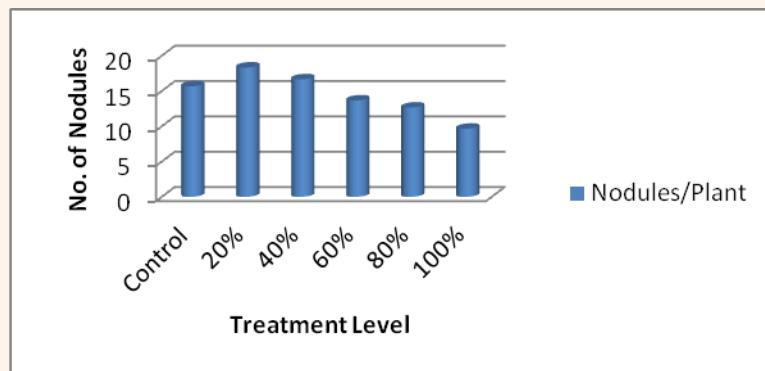
Observation and Results

Table 25: Effect of dyeing and printing effluent on Leghaemoglobin (mg g⁻¹ fresh nodule) of *Medicago sativa* L. after completion of life cycle (60 DAS)

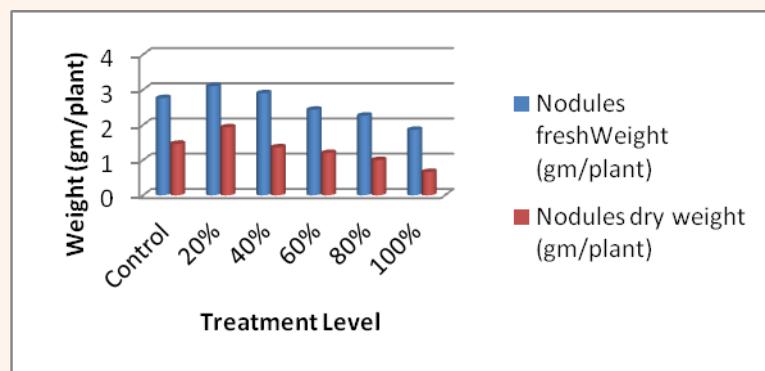
(Values are mean ± Standard Deviation of 3 replicates)

	Leghaemoglobin	
	590 nm.	556 nm.
Control	0.291±0.010	0.204±0.010
T ₁	0.362±0.001 (+24.39%)**	0.229±0.011 (+12.25%)**
T ₂	0.348±0.099 (+19.58%)**	0.215±0.012 (+5.39%)**
T ₃	0.281±0.015 (-3.43%)*	0.177±0.014 (-13.23%)*
T ₄	0.268±0.039 (-7.90%)*	0.156±0.019 (-23.52%)*
T ₅	0.227±0.025 (-21.99%)*	0.139±0.29 (-31.86%)*

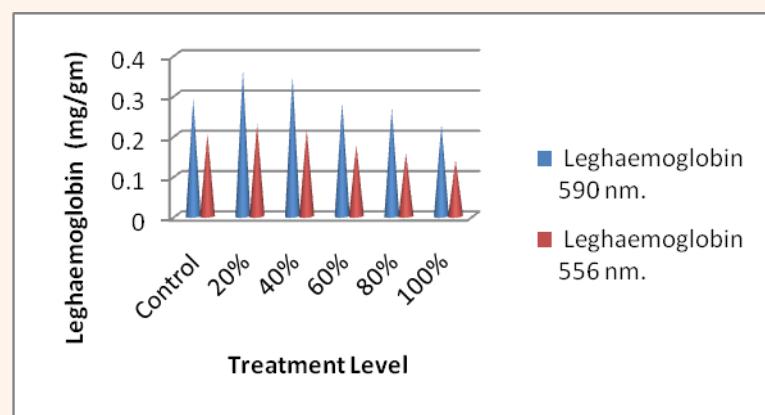
FIGURE - 22



(A) Nodules/Plant



(B) Nodules Weight (gm/plant)



(C) Leghaemoglobin content

Graph showing Effect of different treatment levels of dyeing and printing effluent on nodulation parameters in *Medicago sativa* L. (Pot Experiment)

PLATE - 13



(A) Root system in *Glycine max* L.



(B) Root system in *Medicago sativa* L.

Root System in Both the Experimental Plant

Observation and Results

fresh nodule at 556A) followed by gradual decreasing values in increasing concentration of effluent on treatment level T₃ (60%) 0.28 mg/gm fresh nodule at 590A and 0.17 mg/gm fresh nodule at 556A and T₄ (80%) 0.26 mg/gm fresh nodule at 590A and 0.15 mg/gm fresh nodule at 556A. Reduction in percentage value was found 3.43% at 590A and 13.23% at 556A in T₃ while 7.90% at 590A and 23.52% at 556A in T₄ treatment level in comparison to control.

4.7: Bacterial Population in Soil

4.7.1: Isolation and Identification of Bacteria from soil contaminated with dyeing and printing effluent.

Microbial diversity constitutes the most extraordinary reservoir of life in the biosphere that we have only just begun to explore and understand. The present study was aimed to investigate the bacterial population and bacterial diversity of soil. The soil samples were collected from control site, effluent discharge site and effluent dumping site of dyeing and printing industries at Kaithun region, Kota and effluent treated soil sample from Pot experiment. Samples were used for the isolation of bacterial species using serial dilution and plating methods. Serially diluted sample was poured into the nutrient agar medium showed the number of bacterial species. The results revealed that the bacterial density is high in industrial sites as compared to control (Table 26). It could be due to the higher BOD and COD values of the effluents. Bacterial diversity of soil samples were characterized on the basis of morphological examinations of the obtained colonies depending upon their shape, size, color of background, pigment production and types of colony etc. (Table 27). The polluted soil may contain several types of bacterial species. They were identified by Biochemical test includes Indole, Methyl red, Voges-Proskauer test, Citrate, Catalase and gram staining technique. Three Bacterial isolates such as *Bacillus subtilis*, *Bacillus cereus* and *Pseudomonas aeruginosa* were isolated and identified in contaminated soil of Kaithun region (Table 31).

Observation and Results

Table 26: Microbiological Analysis of Soil

Media Used - nutrient Agar Temperature and Time of incubation - 25 degree centigrade and 48 hours				
Soil Samples from Experimental Sites				
Sample	Dilutions	Dilutions factor	Colony count	No. of Organisms/ ml
Effluent discharge site	10^{-4}	10^4	380	19×10^4
	10^{-5}	10^5	320	16×10^5
	10^{-6}	10^6	280	14×10^6
Effluent dumping site	10^{-4}	10^4	300	15×10^4
	10^{-5}	10^5	260	13×10^5
	10^{-6}	10^6	200	10×10^6
Control	10^{-4}	10^4	320	16×10^4
	10^{-5}	10^5	280	14×10^5
	10^{-6}	10^6	240	12×10^6
Soil Samples from Pot Experiment				
Control	10^{-4}	10^4	220	15×10^4
	10^{-5}	10^5	180	12×10^5
	10^{-6}	10^6	120	10×10^6
T_1 Treatment level	10^{-4}	10^4	280	18×10^4
	10^{-5}	10^5	240	16×10^5
	10^{-6}	10^6	180	13×10^6

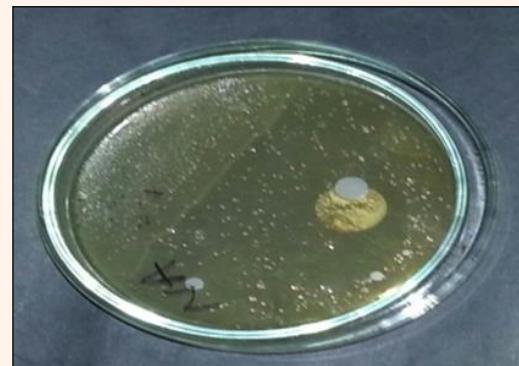
PLATE - 14



(A) Bacterial population at Site A



(B) Bacterial population at Site B



(C) Bacterial population at Site C



(D) Control Soil Samples from Pot Experiment



(E) T₁ treatment level Soil Samples from Pot Experiment

Bacterial Population in Contaminated Soil Samples

Observation and Results

Table 27: Morphological Result

No .	Test	Colony Characters on Nutrient Agar Plate Incubated at 25⁰ C for 48 hours				
		Soil Samples from Experimental Sites			Soil Samples from Pot Experiment	
		Control Site	Main infected Site Sample	Originated Site Sample	Control	T ₁ Treatment level
1	Colony Forms	Punctiform (dot like),Circular, Rizoid	Punctiform (dot like),Circular	Punctiform (dot like), Circular	Punctiform (dot like), Circular	Punctiform (dot like), Circular
2	Color of cell	whitish, yellowish, offwhite ,creamy	whitish , yellowish ,offwhite ,creamy	Offwhite ,creamy	whitish , yellowish ,offwhite ,creamy	whitish , yellowish ,offwhite ,creamy
3	Color of back ground	pasty, offwhite	pasty, offwhite	pasty, offwhite	pasty, offwhite	pasty, offwhite
4	Pigment Production	Negative	Negative	Negative	Negative	Negative
5	Types of colony	4	4	2	2	3

4.7.2.: Biochemical tests for identification of isolate *Pseudomonas aeruginosa*

- A. Indole Test** - The tube containing SIM agar medium at pH 7.3 were inoculated by isolated *Pseudomonas aeruginosa* bacteria, after 24-48 hr of inoculation on addition of Kovac's reagent, no change in color of media was observed showed negative test for indole (Table-31, Plate-15, 23).
- B. Methyl Red** - The tubes containing (MR-VP Broth) at pH 7.3 were inoculated by isolated *Pseudomonas aeruginosa* bacterial culture. After 24-48 hours of incubation on addition of Methyl red reagent, change in color of the medium was observed (Table-31, Plate-15, 23) and found that red color was not appeared showed negative test for MR.
- C. Voges Proskauer**- The tubes containing (MR-VP Broth) at pH 7.3 was inoculated by isolated *Pseudomonas aeruginosa* bacterial culture. After 24-48 hours of incubation on addition of Barritt's reagent A and B, change in color of the medium was observed and found that there was no color change was observed it indicate negative test for VP (Table-31, Plate-15, 23).
- D. Citrate Utilization**- The tube containing (Simmons Citrate Agar) at pH-6.9 were inoculated by isolated *Pseudomonas aeruginosa* bacterial culture. After 24-48 hours of incubation change in the color of the media was observed showed positive test for citrate (Table-31, Plate-15, 23).
- E. Catalase Test**- Slide containing 2-3 drops of (Trypticase soya broth) at pH-7.3 were inoculated by 24-48 hours isolated *Pseudomonas aeruginosa* bacterial culture. After few seconds on addition of 3% hydrogen peroxide observed. Appearance of bubbles showed positive test for catalase (Table – 31, Plate-15, 23).

Observation and Results

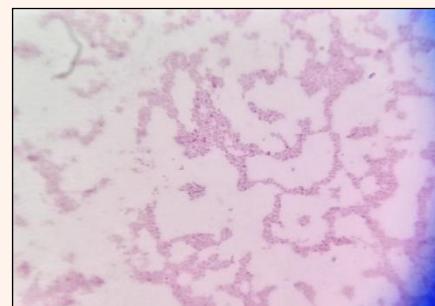
**Table: 28 Biochemical Test and Identification of
*Pseudomonas aeruginosa***

S. No.	Characteristics	Morphological, Cultural and Biochemical Results (<i>Pseudomonas aeruginosa</i>)
1.	Gram Staining	Negative
2.	Shape	Rods
3.	Motility	Motile
4.	Capsule(Capsulated/NonCapsulated)	Non-Capsulated
5.	Spore (Sporing/Non-Sporing)	Non-Sporing
6.	Flagella(Flagellated/Non- Flagellated	Single Flagella
7.	Catalase	Positive (+ve)
8.	Oxidase	Positive (+ve)
9.	MR – Test	Negative (-ve)
10.	VP – Test	Negative (-ve)
11.	Indole	Negative (-ve)
12.	Citrate	Positive (+ve)
13.	H ₂ S	Negative (-ve)
14.	Glucose Fermentation	Negative (-ve)

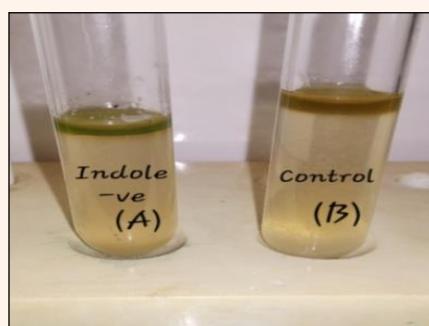
PLATE - 15



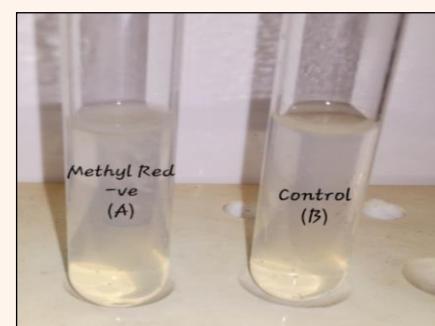
(A) Culture Plate of *P. aeruginosa*



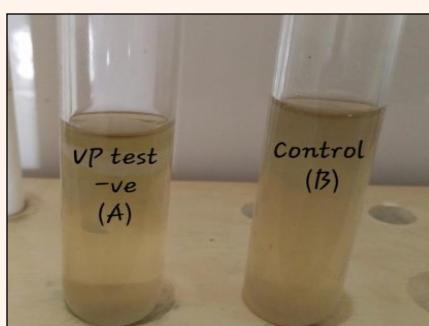
(B) Microscopic View



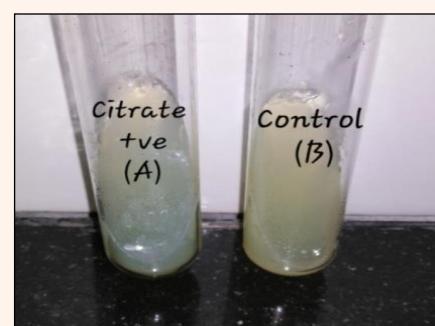
(C) Indole Test



(D) Methyl Red Test



(E) Voges Proskauer Test



(F) Citrate Test



(G) Catalase Test

Biochemical Reaction by *Pseudomonas aeruginosa*

4.7.3: Biochemical tests for isolated *Bacillus subtilis*

- A. **Indole Test** - The tube containing SIM agar medium at pH 7.3 were inoculated by *Bacillus subtilis* isolated bacteria, after 24-48 hr of inoculation on addition of Kovac's reagent, no change in color of media was observed showed negative test for indole (Table-31, Plate-16, 24, 25, 30).
- B. **Methyl Red** - The tubes containing (MR-VP Broth) at pH 7.3 were inoculated by isolated *Bacillus subtilis* bacterial culture. After 24-48 hours of incubation on addition of Methyl red reagent, change in color of the medium was observed, (Table-31, Plate-16, 24, 25, 30) and found that red color was not appeared showed negative test for MR.
- C. **Voges Proskauer**- The tubes containing (MR-VP Broth) at pH 7.3 was inoculated by isolated *Bacillus subtilis* bacterial culture. After 24-48 hours of incubation on addition of Barritt's reagent A and B, change in color of the medium was observed and find that color change was observed showed positive test for VP (Table-31, Plate-16, 24, 25, 30).
- D. **Citrate Utilization**- The tube containing (Simmons Citrate Agar) at pH-6.9 were inoculated by isolated *Bacillus subtilis* bacterial culture. After 24-48 hours of incubation change in the color (blue) of the media was observed. Blue color indicated positive reaction (Table-31, Plate-16, 24, 25, 30).
- E. **Catalase Test**- Slide containing 2-3 drops of (Trypticase soya broth) at pH-7.3 were inoculated by 24-48 hours isolated *Bacillus subtilis* bacterial culture. After few seconds on addition of 3% hydrogen peroxide observe the change on slide. Appearance of bubbles showed positive test for catalase (Table-31, Plate-16, 24, 25, 30).

Observation and Results

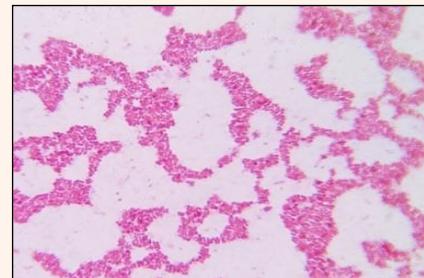
**Table: 29 Biochemical Test and Identification
of *Bacillus subtilis***

S. No.	Characteristics	Morphological, Cultural and Biochemical Results (<i>Bacillus subtilis</i>)
1.	Gram Staining	Positive
2.	Shape	Rods
3.	Motility	Motile
4.	Capsule(Capsulated/NonCapsulated)	Non-Capsulated
5.	Spore (Sporing/Non-Sporing)	Non-Sporing
6.	Flagella(Flagellated/Non- Flagellated)	Flagellated
7.	Catalase	Positive (+ve)
8.	Oxidase	Variable
9.	MR – Test	Negative (-ve)
10.	VP – Test	Positive (+ve)
11.	Indole	Negative (-ve)
12.	Citrate	Positive (+ve)
13.	H ₂ S	Negative (-ve)
14.	Glucose Fermentation	Positive (+ve)

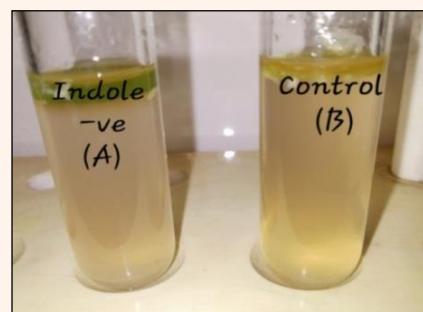
PLATE - 16



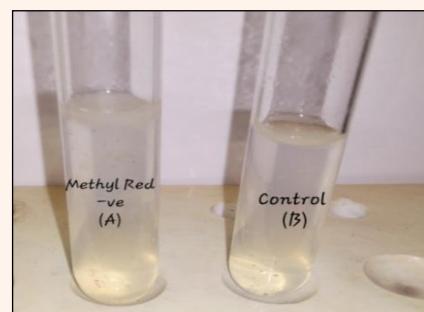
(A) Culture Plate of *B. Subtilis*



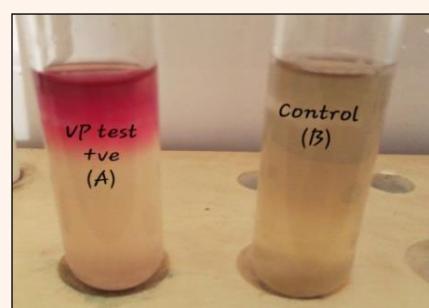
(B) Microscopic View



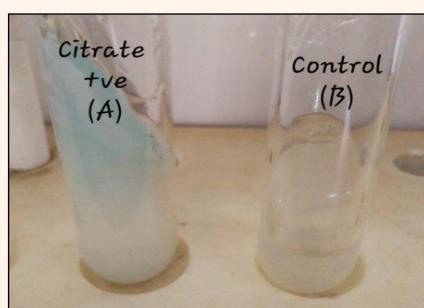
(C) Indole Test



(D) Methyl Red Test



(E) Voges Proskauer Test



(F) Citrate Test



(G) Catalase Test

Biochemical Reaction by *Bacillus subtilis*

4.7.4: Biochemical tests for isolated *Bacillus cereus*

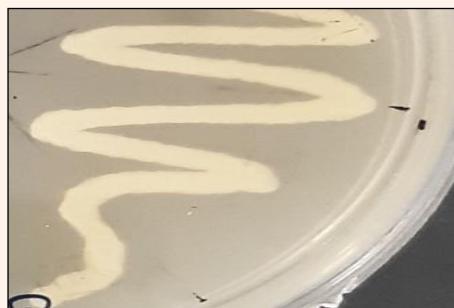
- A. *Indole Test*** - The tube containing SIM agar medium at pH 7.3 were inoculated by *Bacillus cereus* isolated bacteria, after 24-48 hr of inoculation on addition of Kovac's reagent, no change in color of media was observed showed negative test for indole (Table-31, Plate-17, 28, 29).
- B. *Methyl Red*** - The tubes containing (MR-VP Broth) at pH 7.3 were inoculated by isolated *Bacillus cereus* bacterial culture. After 24-48 hours of incubation on addition of Methyl red reagent, change in color of the medium was observed, (Table-31, Plate-17, 28, 29) and found that red color was not appeared showed negative test for MR.
- C. *Voges Proskauer***- The tubes containing (MR-VP Broth) at pH 7.3 was inoculated by isolated *Bacillus cereus* bacterial culture. After 24-48 hours of incubation on addition of Barritt's reagent A and B, change in color of the medium was observed showed positive test for VP (Table-31, Plate-17, 28, 29).
- D. *Citrate Utilization***- The tube containing (Simmons Citrate Agar) at pH-6.9 were inoculated by isolated *Bacillus cereus* bacterial culture. After 24-48 hours of incubation change in the color of the media was observed indicate positive test for citrate (Table-31, Plate-17, 28, 29).
- E. *Catalase Test***- Slide containing 2-3 drops of (Trypticase soya broth) at pH-7.3 were inoculated by 24-48 hours isolated *Bacillus cereus* bacterial culture. After few seconds on addition of 3% hydrogen peroxide observe the change on slide. Appearance of bubbles showed positive test for catalase (Table-31, Plate-17, 28, 29).

Observation and Results

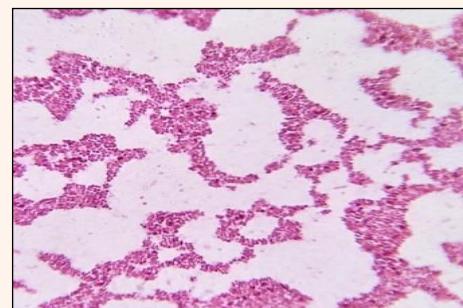
Table: 30 Biochemical Test and Identification of *Bacillus cereus*

S. No.	Characteristics	Morphological, Cultural and Biochemical Results (<i>Bacillus cereus</i>)
1.	Gram Staining	Positive
2.	Shape	Rods
3.	Motility	Motile
4.	Capsule(Capsulated/NonCapsulated)	Non-Capsulated
5.	Spore (Sporing/Non-Sporing)	Sporing
6.	Flagella(Flagellated/Non- Flagellated	Flagellated
7.	Catalase	Positive (+ve)
8.	Oxidase	Negative (-ve)
9.	MR – Test	Negative (-ve)
10.	VP – Test	Positive (+ve)
11.	Indole	Negative (-ve)
12.	Citrate	Positive (+ve)
13.	H ₂ S	Negative (-ve)
14.	Glucose Fermentation	Positive (+ve)

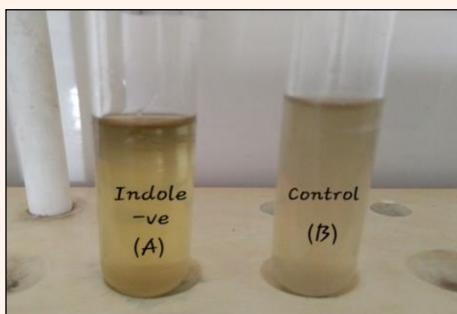
PLATE - 17



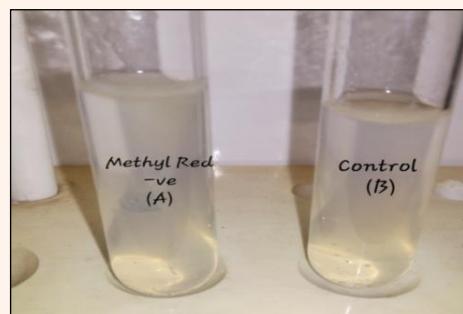
(A) Culture Plate of *B. Cereus*



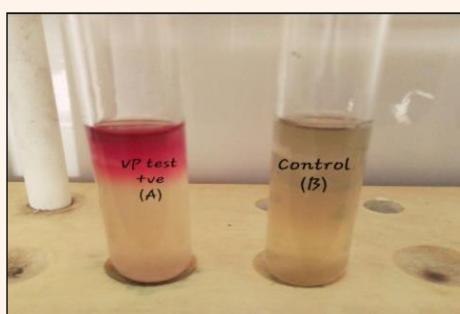
(B) Microscopic View



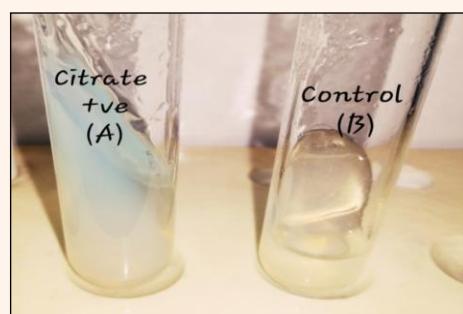
(C) Indole Test



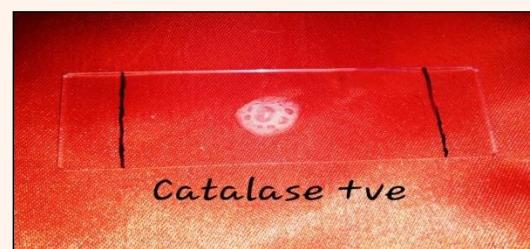
(D) Methyl Red Test



(E) Voges Proskauer Test



(F) Citrate Test



(G) Catalase Test

Biochemical Reaction by *Bacillus cereus*

Observation and Results

Table 31: Morphological, Cultural and Biochemical Characteristics of Isolated Soil Bacteria

Organisms	Morphological, Cultural and Biochemical characteristics of soil bacteria							
	Gram stain	Shape	Growth on Agar	Indole production	MR reaction	VP reaction	Citrate test	Catalase test
<i>Pseudomonas aeruginosa</i>	-	Rod	Adundant thin, white medium turns green	-	-	-	+	+
<i>Bacillus subtilis</i>	+	Rod	Rough, abundant, waxy growth	-	-	+	+	+
<i>Bacillus cereus</i>	+	Rod	Abundant, opaque, white waxy growth	-	-	+	+	+

4.7.5: Isolation and Identification of *Rhizobium* bacteria

Each major legume group is nodulated by different species of *rhizobium*. The mature root nodules were collected from both the experimental plants viz. *Glycine max* L. and *Medicago sativa* L. treated with 20% concentrated effluent (T_1) and control. The prior results revealed that at 20% effluent concentration plant showed positive growth response and nodule development over control. These nodules were sterilized and *rhizobium* were inoculated on YEMA agar, After 24-48 hour inoculation on YEMA plates bacterial colonies were appeared, marked their location and re-streak again to avoid contamination. These isolated bacteria from two different legumes were *Rhizobium japonicum* (*Glycine max* L.) and *Rhizobium meliloti* (*Medicago sativa* L.). These species of *rhizobia* were confirmed on the basis of morphological, cultural and biochemical characters. Colonies appearing white to somewhat translucent circular on the agar surface. Isolated colonies of *rhizobium* were transferred on YEMA medium slopes and stored in the refrigerator for further studies. The results are given in Table 34.

4.7.5.1: Morphological Characters – The *Rhizobium japonicum* and *Rhizobium meliloti* were Gram negative, pink color, aerobic, non spore forming, motile rods (rods were arranged in single, in pairs or in clusters). In general the colonies were circular, convex, whitish pink and glistening with entire margins.

4.7.5.2: Motility Test - The tube contains agar at pH 7.3 were inoculated by isolated *rhizobium* from both legume plants. The pattern of growth in the motility agar stab culture of *rhizobium* of *Glycine max* L. was observed after 48-72 hr of incubation, and observed that bacteria move slowly from the stab line in to medium. *Rhizobium* of *Medicago sativa* L. move slowly but comparative faster than *rhizobium* of *Glycine max* L.

Observation and Results

4.7.5.3: Cultural Characters-Two to three days old culture grown on YEM agar plate examined for colony characters, colonies of *Medicago sativa L* and *Glycine max L*, were circular, convex, whitish pink and glistening with entire margin. (Table-34, plate-19, 20). Slow growing strains of *Glycine max L* produce white, opaque, circular, granular colonies, which do not exceed 1mm in diameter after prolonged incubation. 5-10 days old culture grown on YEMA agar plate examined for colony characters, colonies of *Glycine max L*. were circular, convex, whitish pink and glistening with entire margin. These are slow growing bacteria having more than 12 hr generation time. The colony were not exceed more than 1mm in diameter in 5-7days incubation on YEMA. Three days old culture grown on YEM agar plate examined for colony characters, colonies of *Medicago sativa L* were circular, convex, whitish pink and glistening with entire margin.

Observation and Results

Table 32: Cultural, Morphological and Biochemical Character of *Rhizobium japonicum* (*Glycine max L.*)

Sr. No.	Characters	Result
1.	Shape	Circular
2.	Color	Whitish pink and glistening
3.	Opacity	Opaque/Semitransparent
4.	Margin	Regular/entire
5.	Elevation	Convex/ Raised
6.	Shape	Rod shaped
7.	Oxygen demand	Aerobic
8.	Motility	Motile
9.	Spore formation	Non spore forming
10.	Gram's nature	Gram Negative
11.	Production of Indole from tryptophan	Negative
12.	Methyl red test	Negative
13.	Voges-Proskauer test	Negative
14.	Citrate utilization as source of carbon	Positive
15.	Production of Hydrogen peroxide	Negative
16.	Catalase test	Positive

Observation and Results

Table 33: Cultural, Morphological and Biochemical Character of *Rhizobium meliloti* (*Medicago sativa L.*)

Sr. No.	Characters	Result
1.	Shape	Circular
2.	Color	Whitish pink and glistening
3.	Opacity	Opaque/Semitransparent
4.	Margin	Regular/entire
5.	Elevation	Convex/ Raised
6.	Shape	Rod shaped
7.	Oxygen demand	Aerobic
8.	Motility	Motile
9.	Spore formation	Non spore forming
10.	Gram's nature	Gram Negative
11.	Production of Indole from tryptophan	Negative
12.	Methyl red test	Negative
13.	Voges-Proskauer test	Negative
14.	Citrate utilization as source of carbon	Positive
15.	Production of Hydrogen peroxide	Negative
16.	Catalase test	Positive

4.7.5.4: Biochemical Characteristics of *Rhizobium japonicum*

Biochemical characteristics of the *Rhizobium* isolates were studied using different tests like Indole, Methyl red and Voges Proskauer test, Citrate utilization, Catalase test (Aneja 1996, 2006). The biochemical tests were carried out in medium at 28°C for 48 hours incubation.

- A. *Indole Test*** - The tube containing SIM agar medium at pH 7.3 were inoculated by isolated *Rhizobium japonicum* bacterial culture, after 24-48 hr of inoculation on addition of Kovac's reagent, no change in color of media indicate indole negative. (Table-34, Plate-20).
- B. *Methyl Red*** - The tubes containing (MR-VP Broth) at pH 7.3 were inoculated by isolated *Rhizobium japonicum* bacterial culture. After 24-48 hours of incubation on addition of Methyl red reagent, change in color of the medium was observed that red color was not appeared showed MR negative (Table-34, Plate-20).
- C. *Voges Proskauer***- The tubes containing (MR-VP Broth) at pH 7.3 was inoculated by isolated *Rhizobium japonicum* bacterial culture. After 24-48 hours of incubation on addition of Barritt's reagent A and B, change in color of the medium was observed and find that there was no color change it indicate VP negative (Table-34, Plate-20).
- D. *Citrate Utilization***- The tube containing (Simmons Citrate Agar) at pH-6.9 were inoculated by *Rhizobium japonicum* bacterial culture. After 24-48 hours of incubation change in the color of the media was observed and result indicate citrate positive (Table-34, Plate-21).
- E. *Catalase Test***- Tubes containing 2-3 drops of (Trypticase soya Agar) at pH-7.3 were inoculated by 24-48 hours isolated Rhizobium culture. After few seconds on addition of 3% hydrogen peroxide observe the change on slide. Appearance of bubbles showed catalase positive (Table-34, Plate-21).

4.7.5.5: Biochemical Characteristics of *Rhizobium meliloti*

Biochemical characteristics of the *Rhizobium* isolates were studied using different tests like Indole, Methyl red and Voges Proskauer test, Citrate utilization, Catalase test (Aneja 1996, 2006). The biochemical tests were carried out in medium at 28°C for 48 hours incubation.

- A. *Indole Test*** - The tube containing SIM agar medium at pH 7.3 were inoculated by isolated *Rhizobium meliloti* bacterial culture, after 24-48 hr of inoculation on addition of Kovac's reagent, no change in color of media indicate indole negative. (Table-34, Plate-26).
- B. *Methyl Red*** - The tubes containing (MR-VP Broth) at pH 7.3 were inoculated by isolated *Rhizobium meliloti* bacterial culture. After 24-48 hours of incubation on addition of Methyl red reagent, change in color of the medium was observed that red color was not appeared showed MR negative (Table-34, Plate-26).
- C. *Voges Proskauer***- The tubes containing (MR-VP Broth) at pH 7.3 was inoculated by isolated *Rhizobium meliloti* bacterial culture. After 24-48 hours of incubation on addition of Barritt's reagent A and B, change in color of the medium was observed and find that there was no color change it indicate VP negative (Table-34, Plate-26).
- D. *Citrate Utilization***- The tube containing (Simmons Citrate Agar) at pH-6.9 were inoculated by *Rhizobium meliloti* bacterial culture. After 24-48 hours of incubation change in the color of the media was observed and result indicate citrate positive. (Table-34, Plate-27).
- E. *Catalase Test***- Tubes containing 2-3 drops of (Trypticase soya Agar) at pH-7.3 were inoculated by 24-48 hours isolated *Rhizobium meliloti* bacterial culture. After few seconds on addition of 3% hydrogen peroxide observe the change on slide. Appearance of bubbles showed catalase positive (Table-34, Plate-27).

In both experimental sets of plant *Medicago sativa* L. and *Glycine max* L. (at 20% treatment level) show positive reaction to Citrate utilization, Catalase test, and negative reaction to Indole test, Methyl red test and Voges-Proskauer test.

PLATE - 18



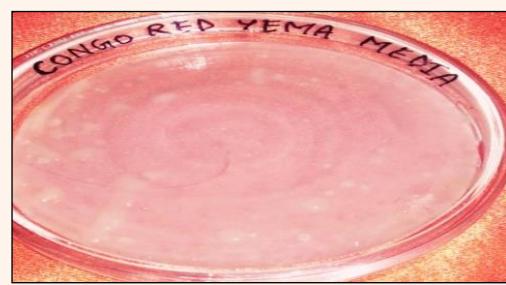
(A) Root nodules from *Glycine max* L.
(Control)



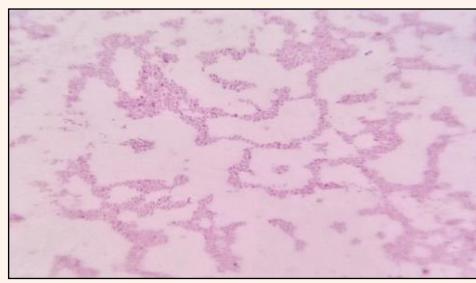
(B) Root nodules from *Glycine max* L.
(20% Treatment Level)



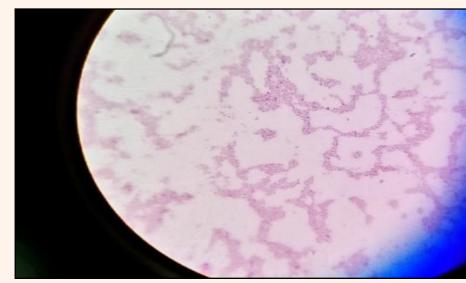
(C) Culture Plate of *Glycine max* L.
(Control)



(D) Culture Plate of *Glycine max* L.
(20% Treatment Level)



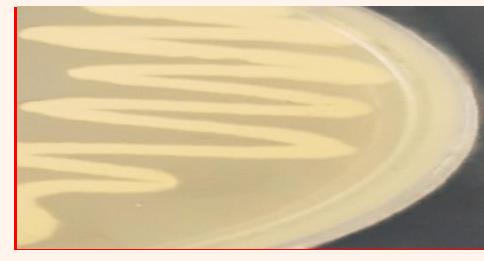
(E) Microscopic view of Bacteria
(Control)



(F) Microscopic view of Bacteria
(20% Treatment Level)



(G) Pure Culture Plate of Bacteria
(Control)



(H) Pure Culture Plate of Bacteria
(20% Treatment Level)

Various Stages of Rhizobium Culture in *Glycine max* L.

PLATE - 19



**(A) Root nodules from *Medicago sativa* L.
(Control)**



**(B) Root nodules from *Medicago sativa* L.
(20% Treatment Level)**



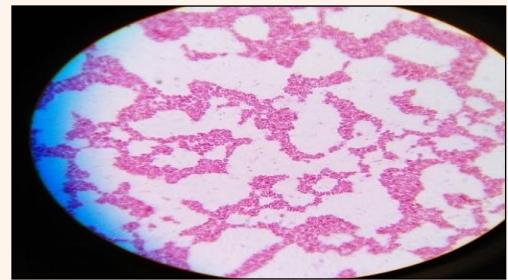
**(C) Culture Plate of *Medicago sativa* L.
(Control)**



**(D) Culture Plate of *Medicago sativa* L.
(20% Treatment Level)**



**(E) Microscopic view of Bacteria
(Control)**



**(F) Microscopic view of Bacteria
(20% Treatment Level)**



**(G) Pure Culture Plate of Bacteria
(Control)**



**(H) Pure Culture Plate of Bacteria
(20% Treatment Level)**

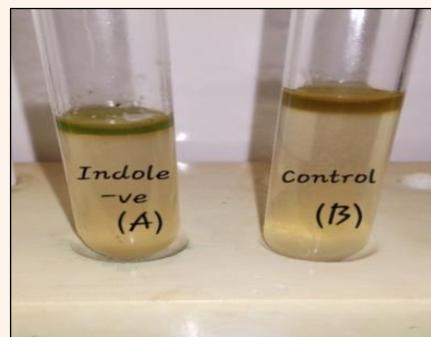
Various Stages of Rhizobium Culture in *Medicago sativa* L.

Observation and Results

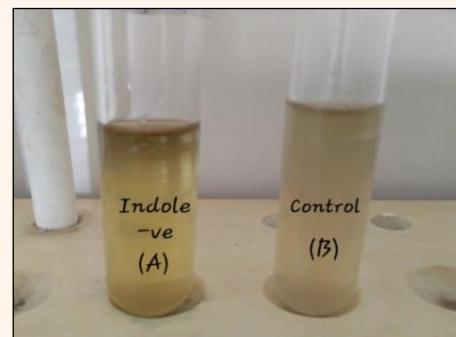
Table 34: Cultural, Morphological and Biochemical Characteristics of Isolated *Rhizobium*:

Sr. No.	Characters	Glycine max L (JS 335) (<i>Rhizobium japonicum</i>)	Medicago sativa L (T9) (<i>Rhizobium meliloti</i>)
1.	Shape	Circular	Circular
2.	Color	Whitish pink and glistering	Whitish pink and glistering
3.	Opacity	Opaque/ Semitransparent	Opaque/ Semitransparent
4.	Margin	Regular/entire	Regular/entire
5.	Elevation	Convex/ Raised	Convex/ Raised
6.	Shape	Rod shaped	Rod shaped
7.	Oxygen demand	Aerobic	Aerobic
8.	Motility	Motile	Motile
9.	Spore formation	Non spore forming	Non spore forming
10.	Gram's nature	Gram Negative	Gram Negative
11.	Production of Indole from tryptophan	Negative	Negative
12.	Methyl red test	Negative	Negative
13.	Voges-Proskauer test	Negative	Negative
14.	Citrate utilization as source of carbon	Positive	Positive
15.	Catalase test	Positive	Positive

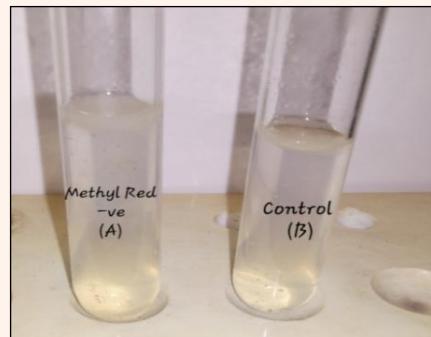
PLATE - 20



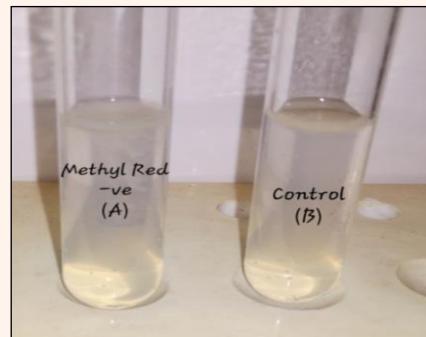
**(A) Indole Test
(Control)**



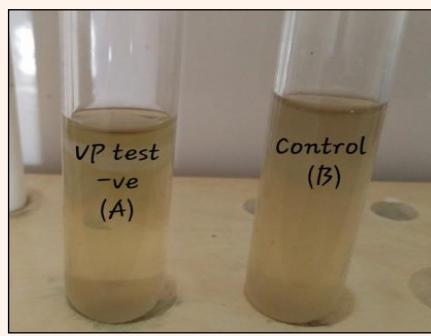
**(B) Indole Test
(20% Treatment Level)**



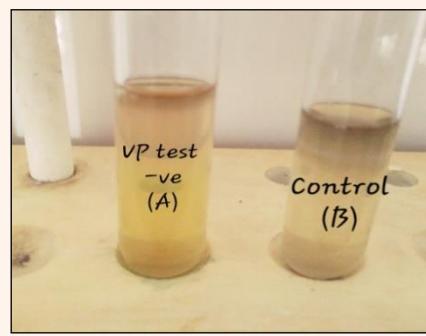
**(C) Methyl Red Test
(Control)**



**(D) Methyl Red Test
(20% Treatment Level)**



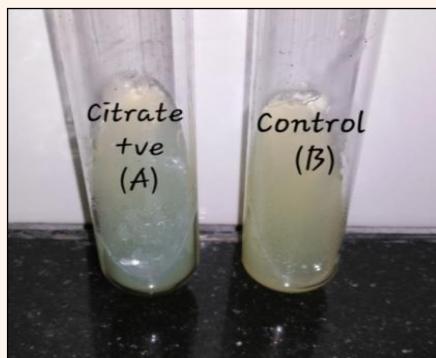
**(E) Voges Proskauer Test
(Control)**



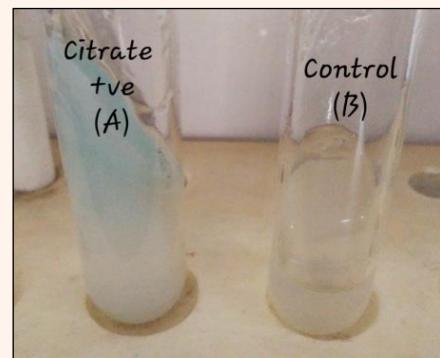
**(F) Voges Proskauer Test
(20% Treatment Level)**

Tubes Showing Biochemical Reaction by Rhizobial Strains of *Glycine max L.*

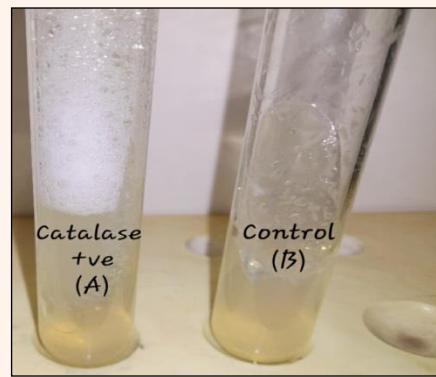
PLATE - 21



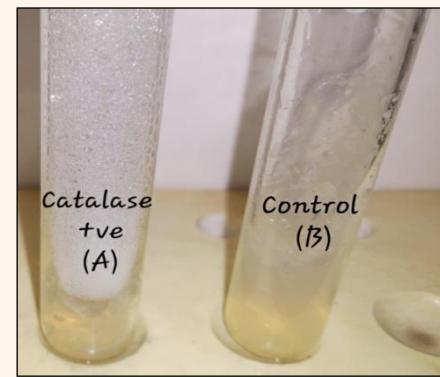
**(G) Citrate Test
(Control)**



**(H) Citrate Test
(20% Treatment Level)**



**(I) Catalase Test
(Control)**



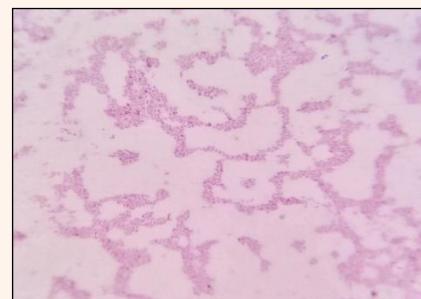
**(J) Catalase Test
(20% Treatment Level)**

Tubes Showing Biochemical Reaction by Rhizobial Strains of *Glycine max L.*

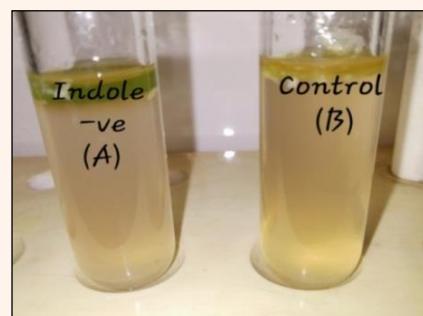
PLATE - 22



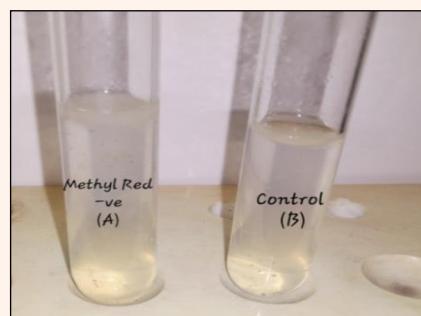
(A) Culture Plate of *B. Subtilis*



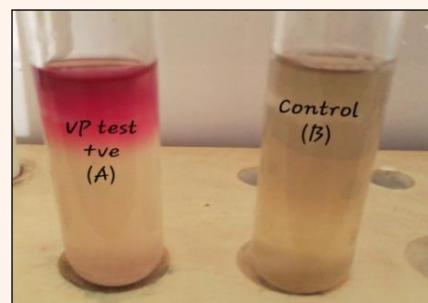
(B) Microscopic View



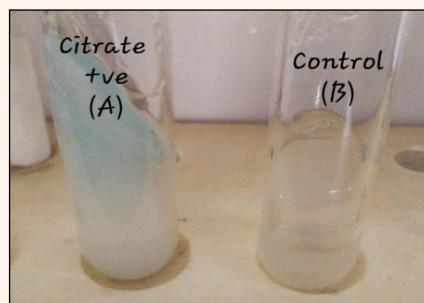
(C) Indole Test



(D) Methyl Red Test



(E) Voges Proskauer Test



(F) Citrate Test



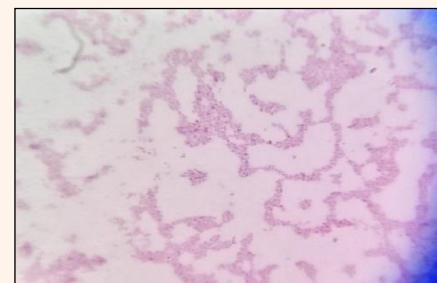
(G) Catalase Test

Tubes Showing Biochemical Reaction by *Bacillus subtilis* from Control treatment level of *Glycine max L.*

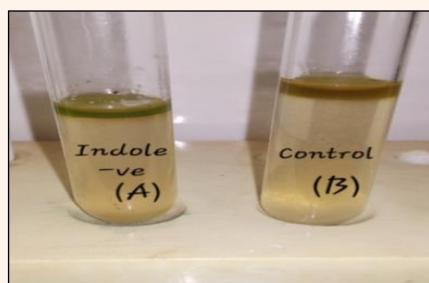
PLATE - 23



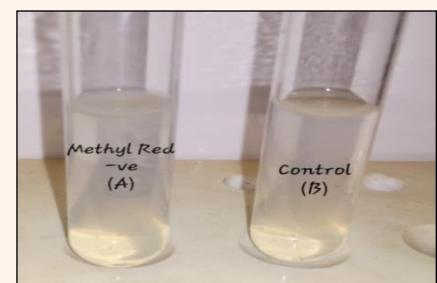
(A) Culture Plate of *P. aeruginosa*



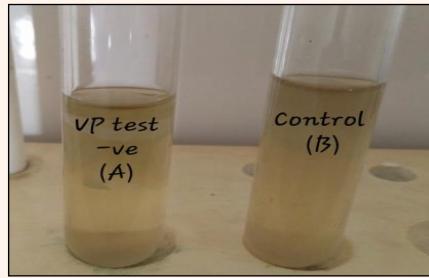
(B) Microscopic View



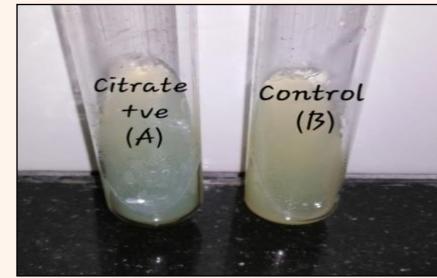
(C) Indole Test



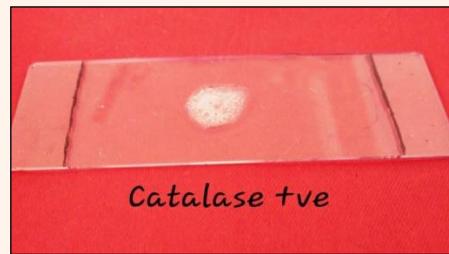
(D) Methyl Red Test



(E) Voges Proskauer Test



(F) Citrate Test



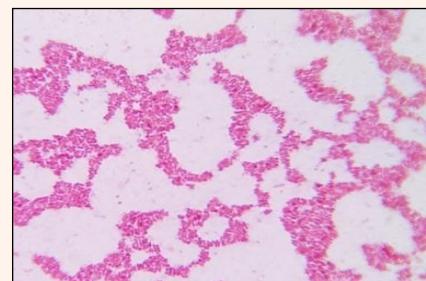
(G) Catalase Test

Tubes Showing Biochemical Reaction by *Pseudomonas aeruginosa* from 20% (T₁) treatment level of *Glycine max L.*

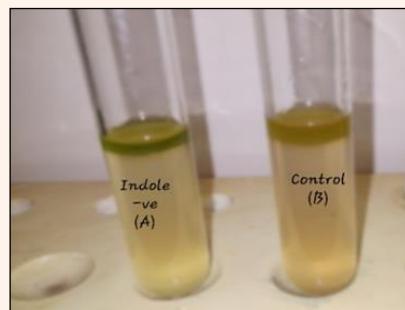
PLATE - 24



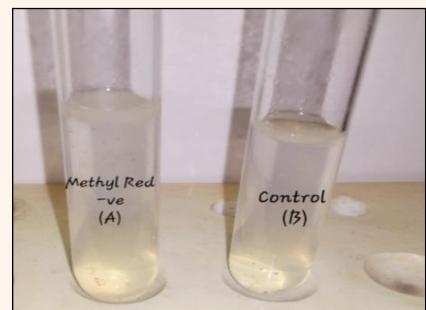
(A) Culture Plate of *B. Subtilis*



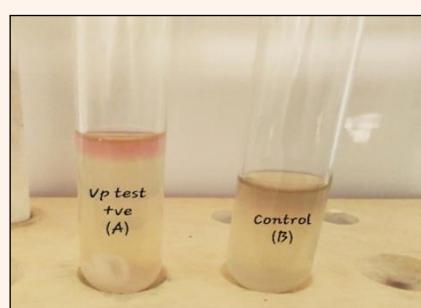
(B) Microscopic View



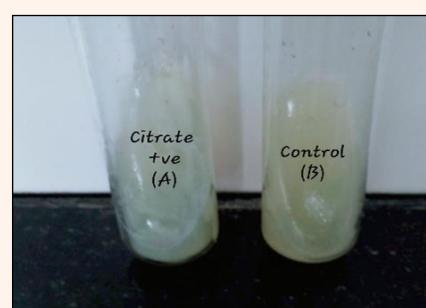
(C) Indole Test



(D) Methyl Red Test



(E) Voges Proskauer Test



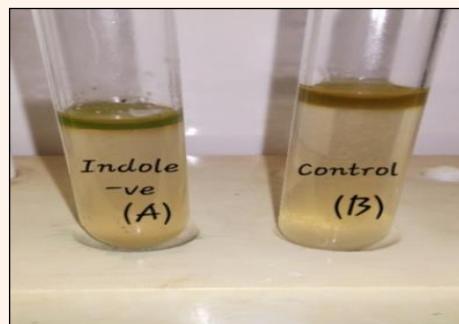
(F) Citrate Test



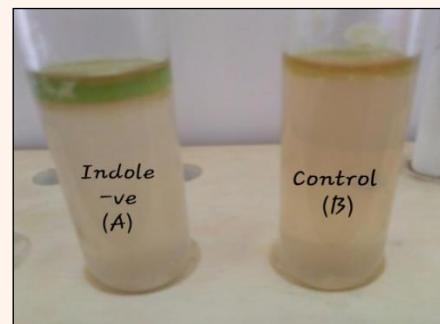
(G) Catalase Test

Tubes Showing Biochemical Reaction by *Bacillus subtilis* from 20% (T₁) treatment level of *Glycine max L.*

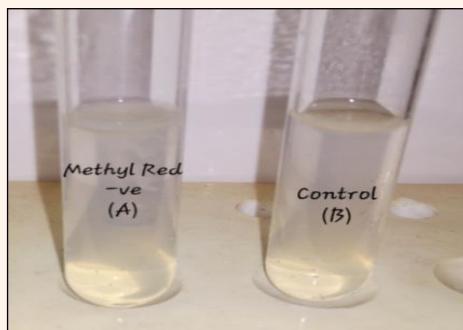
PLATE - 25



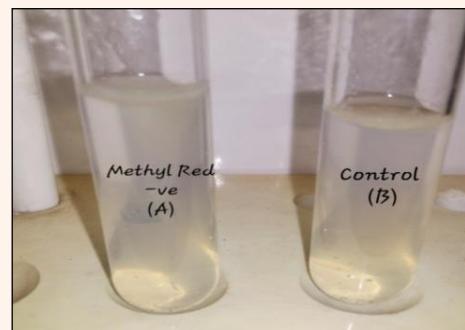
**(A) Indole Test
(Control)**



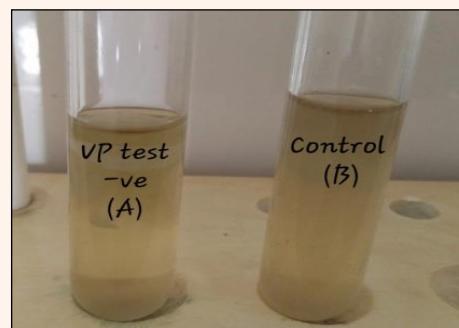
**(B) Indole Test
(20% Treatment Level)**



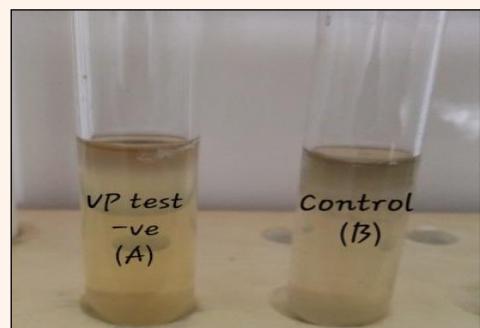
**(C) Methyl Red Test
(Control)**



**(D) Methyl Red Test
(20% Treatment Level)**



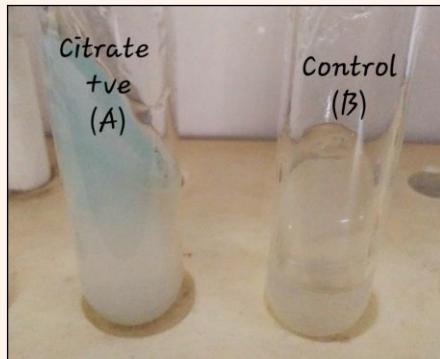
**(E) Voges Proskauer Test
(Control)**



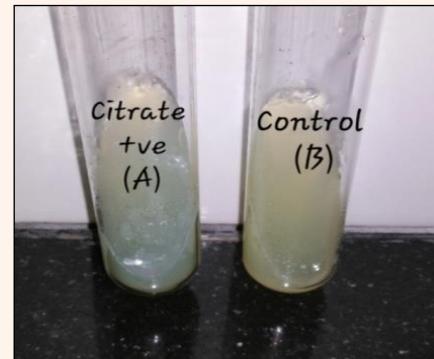
**(F) Voges Proskauer Test
(20% Treatment Level)**

Tubes Showing Biochemical Reaction by Rhizobial Strains of *Medicago sativa L.*

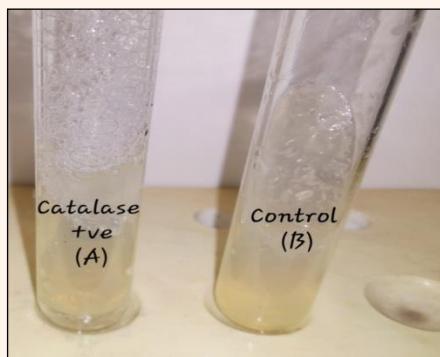
PLATE - 26



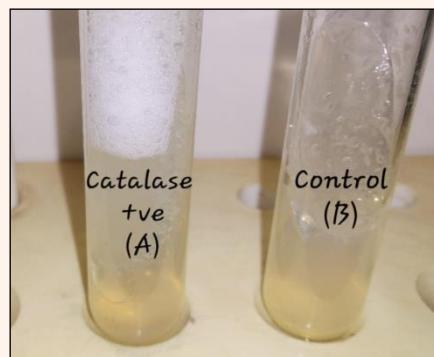
**(G) Citrate Test
(Control)**



**(H) Citrate Test
(20% Treatment Level)**



**(I) Catalase Test
(Control)**



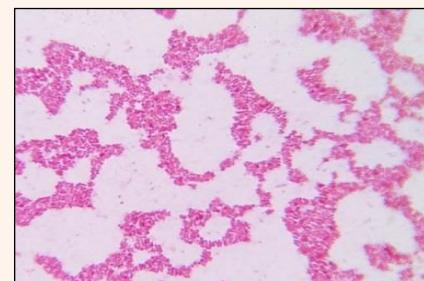
**(J) Catalase Test
(20% Treatment Level)**

Tubes Showing Biochemical Reaction by Rhizobial Strains of *Medicago sativa L.*

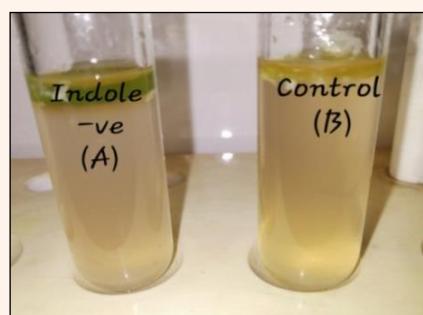
PLATE - 27



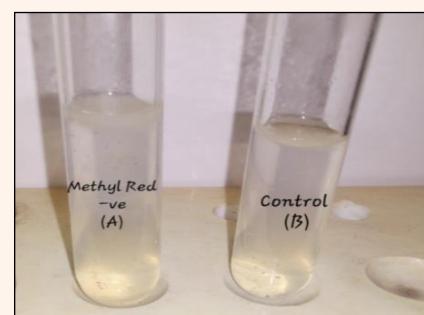
(A) Culture Plate of *B. Subtilis*



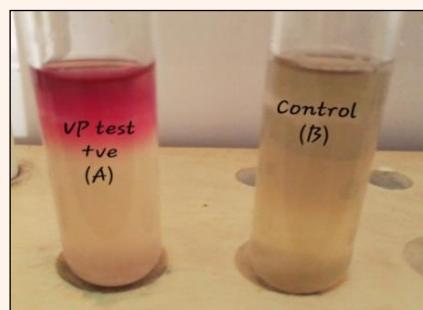
(B) Microscopic View



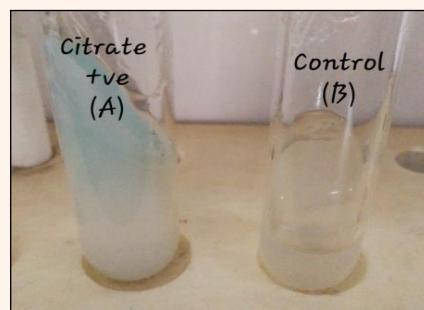
(C) Indole Test



(D) Methyl Red Test



(E) Voges Proskauer Test



(F) Citrate Test



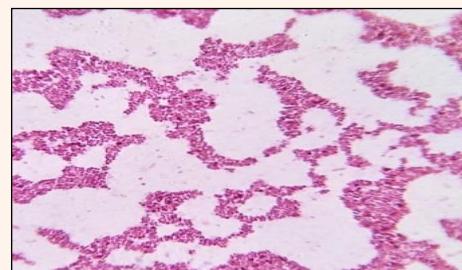
(G) Catalase Test

Tubes Showing Biochemical Reaction by *Bacillus subtilis* from Control treatment level of *Medicago sativa L.*

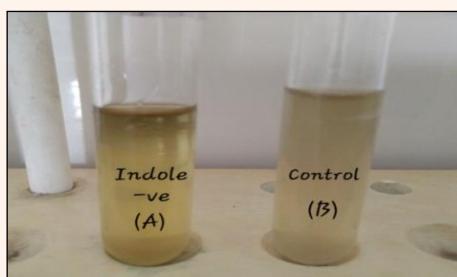
PLATE - 28



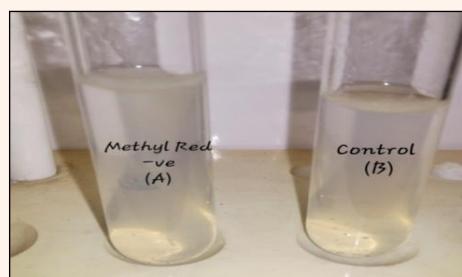
(A) Culture Plate of *B. Cereus*



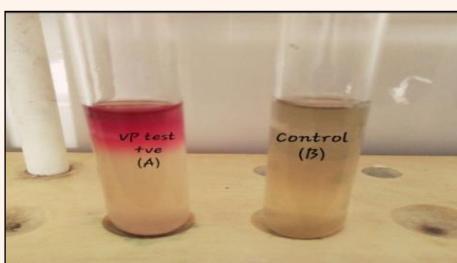
(B) Microscopic View



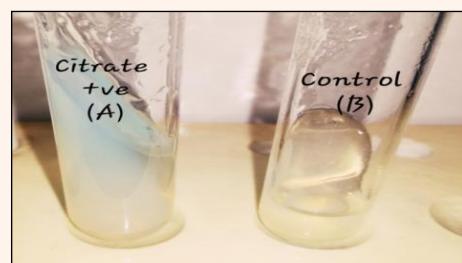
(C) Indole Test



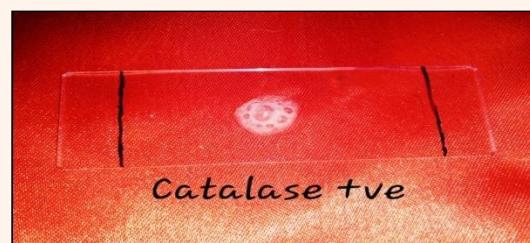
(D) Methyl Red Test



(E) Voges Proskauer Test



(F) Citrate Test



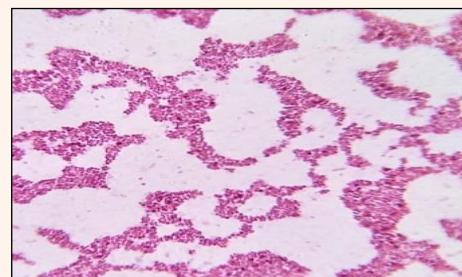
(G) Catalase Test

Tubes Showing Biochemical Reaction by *Bacillus subtilis* from Control treatment level of *Medicago sativa L.*

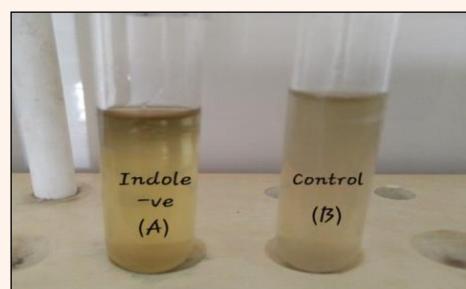
PLATE - 29



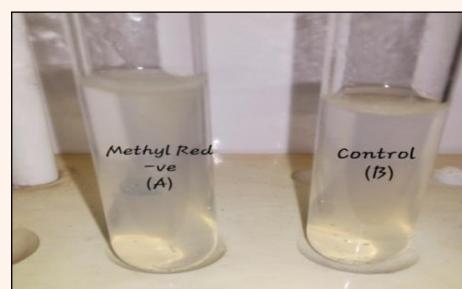
(A) Culture Plate of *B. Cereus*



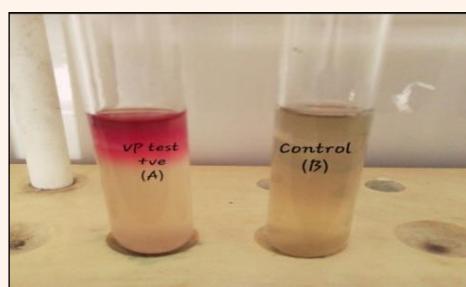
(B) Microscopic View



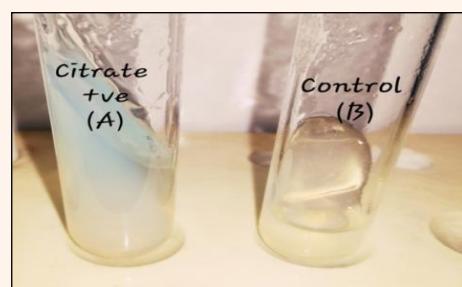
(C) Indole Test



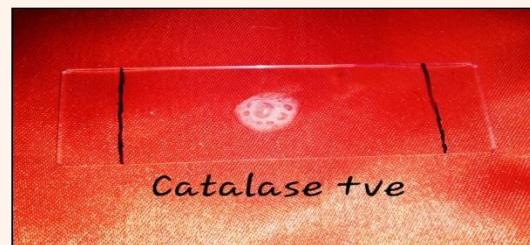
(D) Methyl Red Test



(E) Voges Proskauer Test



(F) Citrate Test



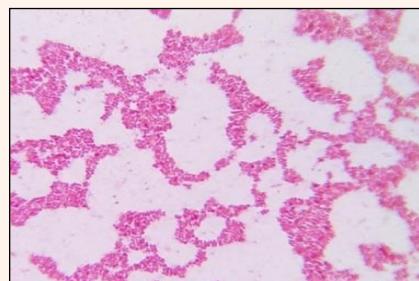
(G) Catalase Test

Tubes Showing Biochemical Reaction by *Bacillus cereus* from 20% (T₁) treatment level of *Medicago sativa* L.

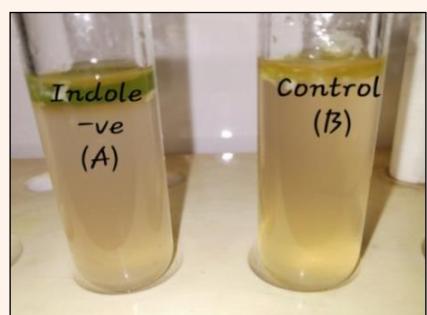
PLATE - 30



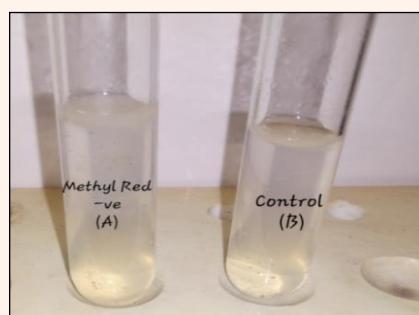
(A) Culture Plate of *B. Subtilis*



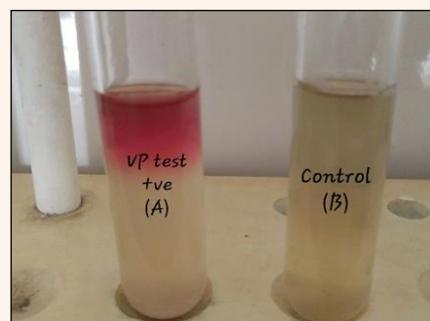
(B) Microscopic View



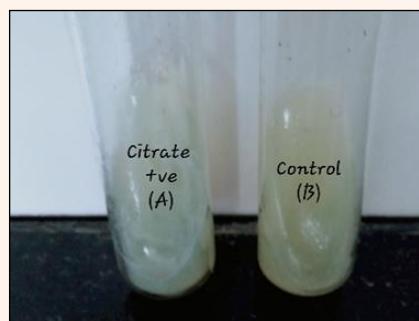
(C) Indole Test



(D) Methyl Red Test



(E) Voges Proskauer Test



(F) Citrate Test



(G) Catalase Test

Tubes Showing Biochemical Reaction by *Bacillus subtilis* from 20% (T₁) treatment level of *Medicago sativa* L.

CHAPTER – 5



DISCUSSION

DISCUSSION

Water pollution due to industrial discharge is obtaining a greater expansion day by day in India. Industries discharge a variety of pollutants with chemical constituents of undesirable concentration which can deteriorate the surface and ground water resources. Industrial wastes generate a large amount of organic and inorganic matters as by-products which when disposed off in river system leads to high BOD and consequent oxygen depletion in the river (IUCN, 2000). Many studies have reported highly toxic effect of industrial effluents to the soil as well as crop productivity whereas few studies have indicated the possibility of using of industrial effluents of certain industries for irrigation (Yousaf *et. al.*, 2010, Mehta, 2012). The industrial effluent can be used for irrigation purpose by the farmers on the basis of the fact that the effluents may serve as a potential source of fertilizer for agricultural use and prevent the wastewater from being an environmental hazard. Use of wastewater in agricultural irrigation is becoming a common and ever increasing practice because of two reasons. Firstly, wastewater represents an extra source of water available for irrigation. Secondly, recycling of the nutrients through the crops and biological degradation of remaining organic matter (Rathor *et. al.*, 2015).

Water is often contaminated by pollutants like fertilizers, pesticides, effluents that are discharged from various industries, sewage and so on (Arul, 2000). Of all the sources of pollutants, the indiscriminate discharge of effluents or spent liquids contributes largely to the quality of ground water. Water pollution is one of the important issues to carryout research and to tackle the problem in different dimensions and also it is necessary to understand the soil properties and crop growth in such affected lands. The pollution of soil is caused by solid wastes, chemical industrial effluent, important ones being textile mill wastes, paper mill wastes, oil refineries, cement industries, plastic and rubber production plants, chemical and fertilizer manufactures, iron and steel plants and others. The industrial effluents definitely affect the soil properties, thereby inhibiting the crop growth in general (Saravanamoorthy and Ranjitha Kumari, 2005). Highly concentrated effluent may have adverse impact on the growth and population of micro and macro

flora of soil and water occur due to disposal of waste water from these industries. Some people used untreated effluent for irrigated purposes or they directly discharge the effluent in soil. The Physico-chemical analysis of the textile dyeing and printing effluent samples gives an idea of the extent type and possible source of pollution and can be used as an argument to emphasize on the treatment of dyeing effluent prior to its discharge on the open land or local water bodies. Many studies emphasized the possibilities of the use of wastewater or industrial effluent as fertilizer or growth stimulant in agricultural field. Sah *et al.* (2000) reported the possibilities of use of wastewater from food processing industries, dairies, breweries, distilleries, paper mills, textile mills and organic chemicals manufacturing industries in irrigation.

(1) Physico-chemical parameters of textile dyeing effluents

Color is a qualitative characteristic that can be used to assess the general condition of wastewater. Color is very important factor for the aquatic life for making food from sun-rays. In fact color in the effluent is easily visible to human eyes even at very low concentration. The color of the dyeing industry effluent of both the sampling site was dark blue and black. According to Elango *et. al.* (2017) dark color of effluent is due to presence of different dyes, colors producing compounds and metals.

The temperature of concentrated effluent of dyeing industry was found within the permissible limit i.e. 26^0C and 31^0C at site A and site B. Similar results were observed by Joshi and Kumar (2011) that the temperature of textile effluent ranged from $25.0\text{-}29.0^0\text{C}$ which was closely similar to this study.

The concentration of hydrogen-ion is a major sign for measurement of quality of natural and waste water (Bharti and Shinkar, 2013). Similar results were observed during the present research work. The effluent of dyeing industry was slightly alkaline in nature with pH value of 7.7 and 7.9 in sample A and Sample B respectively. Result were supported by Nidhi joshi and Ashwani kumar (2011) reported that the pH of textile effluent ranged from 7.6-7.9 whereas Ahmad *et. al.* (2012) reported the pH of dyeing industrial effluent ranged between 8.2 and 9.0. In addition, discharge of untreated dye effluent with either low pH or high pH makes

Discussion

the water unfit for irrigation and the soil over large areas became alkaline resulting in poor crop growth and crop yields. David *et. al.* (2015) found the pH value of effluent samples 7.3 were collected from dyeing industry located at Chinnalapatti, Dindigul district, Tamil Nadu which was very closer to the present study. The result coincides with finding of some other researchers in their studies on the physicochemical characterization of textile wastewater concluded that the wastewater is slightly alkaline, because in most of the steps alkali nature detergents are used in large quantity and The higher value of pH of the textile effluent indicates the alkalinity conditions which have an adverse effect on the soil permeability and soil microflora (Robinson et. al., 2002 ; Shivangi Rana and Krishan kumar, 2017; Prasad A and Rao B.V.,2011; Kumari *et al.* 2011).

Electric conductivity is important for irrigation because it is a measure of the salinity of the water and acts as a surrogate for total dissolved solids (TDS) (Metcalf and Eddy, 2003). In the present investigation the electrical conductivity was 1479 $\mu\text{S}/\text{cm}$ and 2200 $\mu\text{S}/\text{cm}$ at different sampling points which were generally higher than BIS standard 300 $\mu\text{S}/\text{cm}$. Mir Tariq Ahmad *et al* (2012) also studied the physico-chemical characteristics of dye industry effluent and reported that electrical conductivity ranged from 410.38 to 500.46 $\mu\text{S}/\text{cm}$ which was very less than the present study. David *et. al.* (2015) found the Electrical Conductivity in the effluent (2,900 mS/cm) was slightly greater than present study. Sathiyaraj *et. al.* (2017) EC value is found to be higher 8330, 7610, and 7330 $\mu\text{mho}/\text{cm}$ (Erode, Pallipalayam, and Bhavani) compared to unaffected regions which was very higher than present study. Nirgude *et. al.* (2013) reported that EC of effluent from Vapi industrial area Gujarat was ranged between 2900 $\mu\text{S}/\text{cm}$ - 59130 $\mu\text{S}/\text{cm}$. Present research works supports the views of David *et. al.* (2015) but other researchers found electrical conductivity is very high in comparison to the present research work.

COD analysis is useful in pinpointing toxic condition and presence of biological resistant substances (Patel and Pandey, 2008; Suriyanarayanan et. al., 2012). It was observed that the COD values of dyeing effluent were higher than permissible limit (250mg/l) for sample A (6864 mg/l) and sample B (3120 mg/l) in present research work. The COD value of control sample was also calculated i.e. 64 mg/l. Manivasakam (1987) reported that the dye-house effluents showed wide

Discussion

variations in COD (120mg/l - 10,238 mg/l) which were comparatively higher than that of the present study. Manikandan *et al* (2015) reported that the COD of untreated textile effluents were varied from 780 mg/l to 1460 mg/l. Sathiyaraj *et. al.* (2017) reported the COD values of Erode, Pallipalayam, and Bhavani ranged between 609 mg/l -859mg/l. The COD value of present research work is dissimilar with the results of Manikandan *et. al.* (2015); Sathiyaraj *et. al.* (2017). Kaur *et. al.* (2010) worked on industrial effluents from textile industry, Panipat and showed COD ranged between (3893 mg/l – 4691 mg/l) which was very close to the present investigation. Karim *et. al.* (2015) worked on Effluent Treatment Plant (ETP) of K. D. S Textile Mills Ltd., Chittagong and found very low values for inlet 342 mg/l and 214 mg/l for outlet effluent, respectively which differ from the present work.

The Biological Oxygen Demand (BOD) is due to the presence of organic contaminants of dyeing and printing effluents in water bodies. Biological oxygen demand is an overall measurement of the biodegradable organic matter in a wastewater indirectly via microbial oxygen consumption. The low or nil BOD shows good quality water, whereas a high BOD indicates the water is highly contaminated (Sawyer and McCarty, 1978). Biological Oxygen Demand (BOD) value was observed very high than permissible limit (250 mg/l) for sample A (1090 mg/l), but slightly higher in sample B (488 mg/l). Hossain *et. al.* (2014) observed that BOD values of textile effluents were varied between 305.58 mg/l to 608.16 mg/l which is very closely similar to the present study. Varsha *et. al.* (2013) reported that the BOD industry effluent of dye and printing clusters of Bagru region, Jaipur was found in the range of 221 mg/l to 699 mg/l which was within the permissible limit for irrigation and very low in comparison to present study. Ravi *et. al.* (2014) reported 1236 mg/l BOD of textile and dye industry waste effluent. Elango *et. al.* (2017) worked on textile dyeing Effluent of Kongu, Nadu of Tamil Nadu found BOD values were 970 mg/l. Present work supports the view of Ravi *et. al.* (2014) and Elango *et. al.* (2017). The result coincides with finding of some other researchers in their studies on the physicochemical characterization of textile wastewater concluded that the wastewater shows BOD values slightly higher or within the permissible limit (Manikandan *et.al.*, 2015; David *et. al.*, 2012; Smrithi *et. al.*, 2012; Karim *et. al.* 2015; Mehta *et. al.* 2012).

Discussion

Total solid, Total suspended and Total dissolved solid is the measure of total inorganic salts and other substances that are dissolved in water. Present study revealed the TDS value of the raw effluent samples found 980 mg/l for sample A and 1440 mg/l for sample B. The high TDS value may be due to the fixing, bleaching and dyeing agents used for dyeing and printing process. Wins and Murugan (2010) reported high level of total solids (1040 mgL^{-1}) from textile mill effluent which is very closely similar to the present study. Manikandan *et. al.* (2015) reported total dissolved solids in textile industries 6120-13000 mg/l. The high amount of total dissolved solids might be due to the presence of inorganic solvents and dyes in the effluent which were comparatively higher than that of the present study. Kaur *et. al.* (2015) reported that TDS value in textile dyeing effluents (Punjab) ranged between 430-49440 mg/l which were comparatively higher than that of the present study. Similarly some other researchers worked on TDS of textile dyeing and printing effluent and found higher and lower values than the present study (Varsha *et.al.*, 2013; Elango *et. al.*, 2017; Nigude *et. al.*, 2013; Joshi *et. al.*, 2012; Patel *et. al.*, 2015; Hassan *et. al.*, 2013; Mehta *et. al.*, 2004; Ravi *et. al.*, 2014).

In the present study the Calcium hardness and Magnesium hardness were recorded as 800 mg/l and 1450 mg/l respectively for sample A and 110 mg/l and 120 mg/l for sample B, which exceeds the BIS limits. Suriyaprabha *et al* (2018) reported that calcium content in dye industry effluent was in the range of 681.36-1362.72 mg/l which exceeds from the values of present research work. Similar value was obtained by Ahmed *et. al.* (2012) and reported that calcium hardness in textile effluent ranged between 217-420 mg/l and magnesium hardness in textile effluent ranged between 22.81-121 mg/l. Jolly *et. al.* (2012) reported that the magnesium content of untreated and treated dyeing effluent was 0.84-0.72 mg/l which was comparatively lower than present study. Similarly Nirgude *et. al.* (2013) observed that magnesium content in dye industry effluent varies in the range of 1557 mg/l - 11730 mg/l which was found very higher than that of present investigation. The high concentration of magnesium has similar effect on soil and water as calcium. Total hardness of the effluent sample A was very high (2250

Discussion

mg/l) than the sample B (230 mg/l). Manikandan *et. al.* (2015) reported that the total hardness of untreated textile effluents was found to be varied from 1280 mg/l to 3885 mg/l which was comparatively higher than present research work. Similar work on textile effluent was also done by some other researchers and found total hardness values higher or lower than the present investigation (Suriyaprabha *et. al.* (2018); joshi *et. al.*, 2012; Joshi *et. al.* 2012)

Copper in the effluent sample A and sample B was 0.02 mg/l and 0.05 mg/l respectively. Imtiazuddin *et al* (2012) reported that the copper content in textile industry wastewater ranged from 0.07-1.96 mg/l was comparatively higher than that of present study. Similarly Om Prakash Bishnoi and Shikha Roy (2017) reported that the copper content was above the permissible limit (5.78 mg/l) at the spot 2nd but was within the limit at spot 1st (1.75 mg/l) prescribed by IS (3.0 mg/l). Jaishree and T.I. Khan (2013) reported that average concentration of copper was found (4.95 mg/l) in the textile water samples which was comparatively higher than present investigation. The concentration of Zinc in sample A and sample B was 0.31mg/l and 0.39mg/l respectively. Shadma Naaz and Pandey (2010) reported that the nickel in the industrial wastewater (mixture of textile wastewater and sewage) was 1.59 mg/l. Similarly Jaishree and T.I. Khan (2013) reported that average concentration of copper was found (0.045 mg/l) in the textile water samples which was slightly lower than present study. Similarly Imtiazuddin *et al* (2012) reported that the nickel content in textile industry wastewater ranged from 0.75-1.53 mg/l. The value of Iron and Nickel concentration was 0.27mg/l and 0.04mg/l respectively in sample A and 0.21mg/l and 0.01mg/l respectively in sample B. Similarly Imtiazuddin *et.al.* (2012) reported iron and nickel was found between 0.159-0.438 mg/l and 0.133-0.274 mg/l. The heavy metals like copper, zinc, iron and nickel were recorded in very less amount i.e. negligible.

Oil and grease values were found between 21 mg/l – 43.6 mg/l which is quite higher than BIS limit. Elango *et. al.* (2017) recorded 18 mg/l oil and grease in textile dyeing effluent. The presence of oil and grease in water bodies leads to the formation of oil layer, which causes significant environmental problem such as reduction of light penetration into water system therefore photosynthesis process for water living organism is hindered.

Several other researchers analyzed quality parameters for effluents released by various industries (Sugar mill: Arora *et. al.*, 2006; Baskaran *et. al.*, 2009; Samuel and Muthukrarrupan 2011; Paper mill: Himabindu and Reddy, 2005; Sharma *et. al.*, 2005; Dyeing and Tannery industry. Mahale *et. al.*, 1999; Balakrishna *et. al.*, 2008; Dayama *et. al.* 1987; Dhanam 2009; Dairy mill: Bhatnagar and Gupta 2002; Arora *et. al.*, 2005; Uaboi- Egbenni *et. al.*, 2009; Kohle and Pawar 2011 and Power plant: Nagajyoti *et. al.*, 2009). These studies suggested presence of pollutants in varying quantity in effluents and these pollutants are mainly responsible for polluting water streams and soils due to discharge of industrial effluents. The presence of pollutants in varying quantity may be due to the nature of industries and management of the effluents by these industries.

(2) Analysis of soil contaminated by dyeing and printing effluent

On the basis of Physico-chemical analysis of the soil samples, an overview of the effect of textile effluent on the adjoining soil habitat can be obtained. The adverse effects of pollutants from untreated textile effluents on the agricultural soil has been studied and discussed in this research. The soil samples were collected from various impacted area, receiving industrial discharge drain nearby the field. The pH of the all samples was ranged from 7.99-8.19 that was slightly in the alkaline. Similraly, Mathur and Kumar (2013) reported that the pH of the samples was alkaline in nature (8.0-8.8) and higher than that of present study. Our results agree with Shiv kumar *et. al.* (2012) reported that the pH range of all the soil samples in all the industrial area were in the normal range of from 6.2 to 7.9 in Mysore city of India. Similar results were given by Patil *et. al.* (2014) and Om Prakash Bishnoi and Shikha Roy (2017) concluded that effluent impacted soil showed higher pH (alkaline) due to the accumulation of salts with the exposure of the effluent. This might be due to use of higher quantities of basic dyes for dyeing, and use of other alkali based chemicals during different steps of printing and dyeing.

The amount of soluble salts in the soil has directly relationship with the conductivity as it is the current carrying capacity of soil (Ramachandra *et. al.*, 2012). In the present study Electrical Conductivity (EC) ranged from 0.21-0.44 ds/m. Om Prakash Bishnoi and Shikha Roy (2017) reported that the EC values for

Discussion

all the samples were well within the normal range (0-1.5 mmho/cm). Ahmad *et. al.* (2012) reported that Electrical conductivity of the effluent was ranged between 220 to 418 mS/cm in soil samples respectively. Mathur *et. al.* (2013) stated the values of EC ranged from 0.19-0.81 mmhos/cm similar to our result. The high value of electrical conductivity might be due to the presence of high concentration of ions and dyes contributed by numerous printing houses located near the drain. The present investigations on high pH and high EC values of the soil samples were in agreement with the results of the survey conducted by Gupta *et. al.* (1994); Joshi and Kumar (2011).

Soil organic carbon (OC) and organic matter (OM) have long been identified as factors that are important for soil fertility in natural ecosystem (Kucharik *et. al.* 2001). In the present study % organic carbon ranged between 0.53– 0.96% at polluted sites. Similar results were observed by Mojiri (2011) and Mehta and yadav (2013) reported that irrigation with wastewater increases organic carbon content of soil. Similarly, Neetika Mathur and Ashwani Kumar (2013) stated that % organic carbon ranged from 0.18 to 0.24%. Jaishree and T. I. Khan reported that the amount of % organic carbon ranged from 0.24 to 0.42%. Arshi Iram and TI Khan (2018) concluded that Organic matter was varied widely among the various cultivated soils horizons selected for the study ranged between 0.188 to 3.14% i.e. quite higher than the present research work.

In the present study the amount of sodium (Na) in soil contaminated with dyeing and printing effluent was ranged from 2.81-6.22 Kg/ha. Choubey reported sodium value 3.98 Kg/ha to 8.96 Kg/ha. in Gandhisagar reservoir. The sodium content of water is very important to decide its quality for irrigation; salts ultimately affect the soil quality and plant growth. Some researchers reported that sodium directly affects the availability of crop water and causes adverse physico-chemical changes in the soil. (AL-Jasser, 2011; Pescod, 1992; FAO, 1985). Similarly, Rena Mehta and Kanika Yadav (2013) stated that the minimum available sodium was recorded in the control soil which ranged between 35.8 to 56.6 ppm and maximum in effluent soil ranged between 80.3-89.7 ppm. Increase in the sodium ion concentration of soil irrigated with waste water can be attributed to minerals in the waste water (Khai *et. al.* 2008).

Discussion

Phosphorus is considered as the important nutrients that has direct effect on the growth and productivity of plants (Sacks M. and Bernstein N. 2011). In our case the concentration of phosphorus ranged from 16.2-69.3 kg/ha. The available phosphorus ranged between 29 to 36 kg/ha (Mathur and Kumar, 2013) and 28 to 44 kg/ha. (Joshi and Kumar, 2011) in the effluent contaminated soil of sanagner, Jaipur. The present investigation was in agreement with the results of Mathur and Kumar (2013) and Joshi and Kumar (2011). Phosphorus is also a major macronutrient for the growth of the plant but excess amounts of phosphorus enhances the formation of algal blooms in water and depresses the soil quality (Hossain *et. al.* 2014).

Potassium (K) is considered the second important macro element for soil and crop productivity. It is said that potassium normally required for agricultural crop production would be supplied by the effluent (Pescod, M.B., 1992; Ndour *et. al.* 2008). The values of potash concentration ranged from 314-653 kg/ha i.e. higher than the permissible limit. Similar results were observed by Muamar *et. al.* (2014) who revealed that there is increase in value of potassium in the soil irrigated with wastewater (519 ppm) than the other type of soil (115 ppm), results showed that irrigated soil with wastewater contains large amount of Potassium. Neetika Mathur and Ashwani Kumar (2013) reported that values of potash ranged from 190-310 kg/ha. Hossain *et. al.* (2014) concluded that potassium plays an important role in protein synthesis and maintaining water balance in plant. Potassium was reported in range of 378-756 kg/ha and it was found higher than Indian Standard. Increased level of P and K were observed by (Anoop Singh *et al.*, 2002) in leaves treated with paper mill effluent.

Water holding capacity shows physical condition of soil, it is the point at which soil gets completely saturated with water. In the present study water holding capacity of soil ranged between 37.91% - 45.86%. Pollutants and industrial discharge increase the soil water holding capacity (Sheikh and irshaad 1980; Rai *et. al.* 2011). More water holding capacity shows the good physical condition of soil. Use of waste water in agriculture increases the water holding capacity, WHC ranged between 53% to 65% in contaminated soil. Contaminated soil has more water holding capacity than uncontaminated soil reported by Sofee R.E. (1995).

Calcium and magnesium are very important elements for plant life. Present result showed the calcium and magnesium was ranged between 2.9-3.6 gm/l and 3.4-4.0 gm/l respectively in the contaminated soil effluent discharge site and control. According to Ahmad *et. al.* (2012) calcium and magnesium was higher in contaminated soil. The calcium in contaminated soil was ranged between 189 to 273 mg/kg and magnesium was ranged between 8.50 to 45.9 mg/kg. In uncontaminated soil, calcium was ranged from 63 to 94.5 mg/kg and magnesium from 3.08 to 6.99mg/kg. In the Soil of Nagchoon Pond Khandwa, MP, India calcium value ranged from 76.80mg/lit. to 43.00 mg/lit and magnesium ranged from 13.56 mg/lit. to 5.34 mg/lit reported by Saroj Mahajan (2014).

Heavy metals:

The analysis of heavy metal ions i.e. Zn, Fe. Cu and Mn of polluted site revealed that the values are higher than permissible limit prescribed by ISI for industrial effluent. The concentration of heavy metal Zn, Fe. Cu and Mn were found 6.88 ppm, 9.02 ppm, 9. 37 ppm and 11.50 ppm respectively. Presence of heavy metals arises from material used in the dyeing process or in a considerable amount from metal complex dyes (Correia *et. al.*, 1994 and Heinfling *et. al.*, 1997). In the present investigation these metal ions were in permissible limit in effluent but due to irregular and long term discharge of effluent on the soil can increases heavy metal accumulation in soil. Present investigation supports the view of (Narwal *et. al.*, 1993, Brar and Arora, 1997 and Olaniya, 1998)

(3) Effect of dyeing effluent on Germination and seedling growth

During present research work our attempt is to use dyeing and printing effluent from Kaithun Reagion, Kota, to study the effect of various concentrations of dyeing and printing effluent on two selected legumes viz. *Glycine max* L. and *Medicago sativa* L, by conducting the germination experiments, by ‘Petri plate method’ and by ‘Pot experiments’ in natural conditions. To study the effect on physical, biochemical and microbiological parameters data were collected and analyzed.

Seed germination percentage of control and different concentrations of effluent treated both legume plants were given in Table 8 and 17. In *Glycine max* L. and *Medicago sativa* L., the percentage of seed germination was enhanced in 20% treatment level over control. The seed germination percentage was decreased with the increasing concentration of the effluent (above 40%). Our findings are in agreement with Rathor *et. al.* (2015) who reported that the best germination and seedling growth was observed in 25% concentration with growth promoting effect and significantly better than control. Beyond 25% effluent germination percentage and seedling growth decreased gradually. Present study supported with the views of Sundaramoorthy *et. al.* (2000) who investigated that the percentage of seed germination and seedling growth was maximum at 10% diluted effluent than the control while undiluted effluent showed inhibitory effects. Sasikala *et. al.* (2013) studied the impact of dye effluent at various concentrations (4%, 8%, 10%, 12% & 16%) on seed germination of black gram for a period of 15th days. She reported gradual decrease in the shoot and root length of the seedlings with the increase in the dye effluent concentrations. Ramya *et. al.* reported that seed germination and seedling growth in *Arachis hypogaea* L. Var. K6 were gradually decreased with increase in textile effluent concentrations but the best seedling germination and growth was observed in treated effluent and followed by 25% effluent than all other concentrations of effluent. Present research work supports the view of Rathod *et. al.* (2015); Ramya *et. al.* (2017); Mohammad and Khan (1985).

Kannian (2001) reported that lower concentrations of the textile industrial effluent promote the peanut germination at 50% dilution which differs from the present investigation. The reduction in germination percentage at higher concentration may be due to the excess amount of minerals and nutrients present in the effluent (Kumar 1999). Our results are consistent with the findings of other workers (Neelam and Sahai, 1988; Mohammad and Khan, 1985; Sahai *et. al.*, 1983).

(4) Effect of dyeing effluent on Seedling growth

Effect of different levels of dyeing and printing effluent under Petri-plate experiment have shown that the root length was higher in the 20 and 40%

Discussion

treatments level in both the plants. Root length, Shoot length, fresh and dry weights of root and shoot, vigour index were reduced in the plants treated with higher concentration of effluent (60%, 80% and 100%). The observations were taken during seedling growth (Petri plate experiment), 30 and 60 days after sowing (Pot experiment). Results are presented in Table 6,7,8,15,16 and 17. The present investigation revealed that all the above said parameters were enhanced at 20% and 40% (*Glycine max L.*) and at 20% (*Medicago sativa L.*) treatment levels over control. Mohammad and Khan (1985) reported adverse effects of textile effluent (75 & 100% concentrations) on the germination of *Phaseolus aureus* and *Abelmoscus esculentus* seeds, while there was no effect up to 50% concentration of the same effluent. Somashekhar *et al.* (1984) reported around 40 to 75% inhibition in Bajra and Jowar and around 90% inhibition in paddy crops growth with response to textile mills effluents.

Kathirval (2012) estimated that the effect of dye factory effluent was studied with respect to germination and growth of Bengal gram (*Cicer arietinum*). In lower concentration the germination percentage and growth are relatively higher than the control, but gradual decrease in the germination of seeds, seedling growth with increasing in effluent concentration was observed. According to him dye effluent can be safely used for irrigation purpose with proper treatment and dilution at 20%. Rathod *et. al.* (2017) studied the effect of textile effluent with respect to germination and growth of chickpea (*Cicer arietinum L.*) and reported that the best germination and seedling growth was observed in 25% concentration with growth promoting effect and significantly better than control. Beyond 25% effluent, root and shoot length decreased. Murkumar and Chavan (1987) have reported that the higher concentration of effluent decrease enzyme dehydrogenase activity that is considered as one of the biochemical change which may have disrupt germination and seedling growth. Parameswari (2014) also reported the effect of textile and dye effluent on germination and growth parameters of greengram, blackgram and redgram. Diluted textile effluent with water in 1:3 ratio did not have any adverse effect on growth and vigour index of field crops. Growth parameters like germination percent, root length, shoot length and dry matter production increased when the concentration of the effluent decreased.

Discussion

The present investigation might be related to reduction in seedling (root and shoot) lengths with the elevated amounts of total dissolved solids at higher concentrations. This could also be related to the fact that some of the nutrients present in the effluents are essentials but at above level of a particular concentration, that was become hazardous (Hassan *et. al.*, 2013). The presence of optimum level of nutrients in the lower concentration of dye effluent might have increased the Fresh Weight and Dry Weight of crop plant. The reduction in dry weight of plant material may be due to the poor growth under effluent irrigation (Balashouri and Prameela Devi., 1994). The high yield of plant at lower concentration might depend on the enhanced low concentration of pigments, sugar and protein (Pragasam and Kannabiran, 2001). The decreased in shoot length, root length, fresh weight and dry weight were recorded. It may be due to the presence of toxic pollutants in the effluent. The same result affects the respiration of the root (Singh *et. al.*, 1985). The wheat plants irrigated with treated effluent of 2.5-5% showed increases in their heights, leaf areas, seed dry weights, root dry weights, number of seeds, seeds weights compared to the control plants (Jolly *et. al.*, 2008). Similar results were made by Lenin *et al.*, (2014) who reported that the best germination of seedling growth, root length, shoot length, fresh weight and dry weight and tolerant variety were observed with 20% effluent concentration of sago factory effluent with growth promoting effect significantly better than control. Beyond 20% effluent concentration, root and shoot length were reportedly decreased. Present study also supports the view of Chinnusamy *et. al.*, (2001) who observed that root length, shoot length, fresh weight root and shoot, dry weight of root and shoot, germination relative index, vigour index and chlorophyll content were higher in 25% than 50% over control.

Medhi (2011) studied the effect of dye factory effluent at 20% dilution and reported that yield characters are relatively higher than the control, but gradually decrease with the increase in effluent concentration. Saravananmoorthy and Ranjitha Kumari (2007) reported that the increase in shoot and root dry weight was observed at 25% effluent treatment in textile effluent treatment could increase the yield of the plants at 25% and 50% treatments. However, the yield decreased in 100% concentration treatment. Sahar *et. al.* (2017) observed that effect of dilution textile

wastewater on the yield parameters and N, P, K content of the wheat is stimulatory rather inhibitory. Minimum reduction in yield parameters and N, P, K content of of the wheat is stimulatory rather inhibitory. Minimum reduction in yield parameters of wheat was recorded on application of 20% and 30% diluted textile wastewater. Similar results were observed during the present research work.

(4-A) Chlorophyll

The Chlorophyll a and Chlorophyll b content were significantly higher at 20% concentration of effluent and gradually decrease with the increasing concentration of effluent. Ramasubramanian *et. al.* (1993) studied the impact of dye industrial effluent on *Phaseolus mungo* L. and reported a declining trend in pigment content. The same was reported in *Vigna mungo*, by distillery effluent. Similar reduction in pigment level was observed in many plants by various industrial effluent irrigation. In this study the increase of protein, carbohydrate might be due to the increase of chlorophyll pigments, because of the optimum level of chemicals like sulphate, chlorides, calcium, potassium, phosphorous and magnesium in textile industrial effluent.

Present investigation disagree with the findings of Saravamoorthy and Ranjitha Kumari (2007) who reported that the chlorophyll a and chlorophyll b contents were significantly increased at 50% and 75% concentration in peanut varieties.

The chlorophyll a and chlorophyll b contents were increased at 20% and 40% concentration and decreased at higher concentrations in both the experimental plants. This result was supported by the results of Kannian (2001) in 50 and 75% diluted textile industrial effluent, which enhanced the chlorophyll content in groundnut. Anoop Singh *et. al.* (2002) also observed more chlorophyll content in wheat leaves at 50% effluent irrigation over control, while 100% effluent irrigation resulted in reduction of the same.

Mishra and Bera (1995) have also reported that leaf chlorophyll and leaf area were reduced in higher concentrations of effluent, while these parameters were enhanced at lower concentrations. Chlorophyll content indicates the rate of

photosynthetic activity of the plant, which in turn reflects upon the nutrient intake of the plant. Chlorophyll a, b and total chlorophyll contents of plants increased based upon the concentrations of effluent.

Reduction in chlorophyll content induced by effluent may be associated with the mineral ions (Gadallah, 1999). Chlorophyll a, b and total chlorophyll and carotenoids were decreased at increased concentrations of the effluent. The carotenoid content was increased up to 50% concentration of effluent (Krishna and Leelavathi, 2002). This might be due to enhancing influence of increased nitrogen on carotenoid synthesis (Cottenie, 1973).

(4-B) Free Amino acid, Protein and Free sugar

Biochemical changes were analyzed in peanut crops, 50% diluted textile effluent increased the seed germination, total sugars, amino acids, phenol and proline starch, protein and chlorophyll than control (distilled water) of peanut seedlings. These studies showed that effects of an industrial effluent vary from crop to crop. So it is essential to study the effect of industrial effluents on individual crops before their disposal in agricultural fields (Swaminathan and Vaidheeswari, 1991).

The higher concentration of dyeing effluent was significantly decrease amino acid, protein and free sugar of *Glycine max* L. and *Medicago sativa* L. In *Glycine max* L., at T₁ treatment level the values of amino acid, protein and free sugar was maximum which was 43.93%, 5.29% and 8.06% increase over control. While the lowest value of amino acid, protein and free sugar was observed in 100% untreated effluent (2.52 mg/gm, 7.22 mg/gm and 10.40 mg/gm respectively). The reduction in percentage was 65.28%, 45.34% and 6.99% as compared with control. In *Medicago sativa* L., the lowest value of amino acid, protein and free sugar was observed in 100% untreated effluent (5.91 mg/gm, 12.23 mg/gm and 9.66 mg/gm respectively). The reduction in percentage was 11.12%, 6.42% and 11.70% as compared with control. At T₁ (20%) treatment level the values of amino acid, protein and free sugar was maximum which was 7.51%, 1.07% and 3.29% increase over control.

Discussion

Similar results were observed by Ramya *et. al.* (2017) who reported that the textile dye effluent was significantly decreased amino acid and soluble proteins of groundnuts except treated effluent. Highest value of amino acid was observed in treated effluent (83.62), followed by 25% (64.87), 50% (53.62), 75% (48.57) and 100% (26.75) except control. Similarly, the protein content was decreased in all concentration of dye effluent compared to control, followed by treated effluent. The amount of carbohydrate, protein and total free amino acids were comparable with control, their amounts were increased in the 30% effluent treated seeds (Divyapriya *et. al.*, 2014). Similar study was observed by Vijayaragavan *et. al.* (2011) on effect of sugar mill effluent on growth and biochemical contents of *Raphanus sativus* L. The sugar content, amino acid and protein content were higher at low (20% and 40%) concentration of sugar mill effluent in the soil than in the control plants. Further, the values decreased with a gradual increase in effluent (60%, 80% and 100%) concentration. Plants treated with higher effluent concentrations (above 40%) showed lower amounts of amino acid and protein content due to the presence of higher magnesium concentrations and the acidic pH of the effluent. The significant increase in the protein content of plant might be due to the potassium and nitrate in their optimum quantity present in the lower concentration of the effluent as reported by (Kadioglu and Algur, 1990) in pea plants.

Use of effluent for irrigation purpose after diluting with normal water may be beneficial for crop growth and yield even more than the normal water irrigation. The textile industrial effluent irrigated wheat plants produced more seeds, which continued relatively reserved in terms of protein, carbohydrate and lipids. This might be due to the availability of more nutrients in the effluent as compared to the normal water (Anoop Singh *et al.*, 2002).

Swaminathan and Vaidheeswarn (1991) analyzed biochemical changes in peanut crops, and observed that 50% diluted textile effluent increased the seed germination, total sugars, amino acids, phenol and proline starch, protein and chlorophyll than control (distilled water) of peanut seedlings. These studies showed that effects of an industrial effluent vary from crop to crop. So it is essential to study the effect of industrial effluents on individual crops before their disposal in agricultural fields.

Extensive efforts have been made by workers to find out proper dilution for different industrial effluent, which can be used for irrigational purpose (Sahai *et. al.*, 1983; Chaudhary *et. al.*, 1989; Bhatanagar *et. al.*, 1986; Sisodia and Bedi, 1985; Srivastava and Sahai, 1987). Stimulation in growth behaviour at lower concentration has been reported and it has been suggested that after proper dilution, effluent can be used for irrigation purpose.

(4-C) Nodulation

Root nodules are the important sites for conversion of atmospheric nitrogen into ammonia in a legume symbiotic relationship. In the pots small nodules can be seen 2-3 weeks after planting, depending on legume species. They are usually white or gray inside when nodules are young and not fixing nitrogen. As nodules grow in size, they gradually turn pink or reddish in color, indicating nitrogen fixation has started. With the completion of life cycle, it was observed that nitrogen fixation starts with the formation of a nodule after one month; small nodules are visible with the naked eye. The pink or red color is caused by leghaemoglobin that controls oxygen flow to the bacteria. Leghaemoglobin content in nodules is closely related to the amount of nitrogen fixed by the legume–rhizobium symbiosis.

In present study it was observed that root system of plant was highly developed in *Glycine max* L. and *Medicago sativa* L. Results showed that lower concentration (20%-40%) of dyeing effluent beneficial for plant growth as well as development of nodules. Higher concentration (above 40%) of effluent have negative effect on plant and nodules growth. During the present research work root nodules characteristics like number of nodules/plant, fresh and dry weight of nodules, leghaemoglobin content and isolation and identification of Rhizobial strains were also observed. The number of nodules/plant was ranged between 10.3 - 25.3 (*Glycine max* L.) and 9.66 – 18.33 (*Medicago sativa* L.). Kumari *et. al.* 2010 revealed that the number of nodules increased during vegetative phase and decline during flowering and pod setting phase, was also reported by Arya and Singh (1996) in horse gram. Thakur and Singh (2014) studied on effect of Cd on nodulation and leghaemoglobin in soybean and chickpea and found that number of nodules decrease 30%, 35% and 74% (pod filling stage) at 4 μ M, 20 μ M and 40 μ M Cd treatment levels respectively in soybean plant. Thakur and Singh (2014) also reported that Lb content of nodules decrease 15%, 39% and 54% (pod filling

stage) at 4 μM , 20 μM and 40 μM Cd treatment levels respectively in chickpea plant. In the present study also Lb content decreased with the increasing concentration of dyeing and printing effluent. In *Glycine max* L. and *Medicago sativa* L. maximum Lb content was observed at T_1 (20%) treatment level i.e. 0.385 μM and 0.362 μM respectively whereas minimum Lb content was recorded at T_5 (100%) treatment level i.e. 0.257 μM and 0.227 μM respectively. A considerable reduction in Lb content of nodules is the result of degradation of haeme-protein and an initiation of early nodular senescence as also reported earlier (Saraswat S, Rai JPN., 2011).

(4-D) Bacterial Population

Soil formation is the result of the combined action of weathering and colonization of geologic material by micro flora. Soil microorganisms are important because they affect the soil's physical, chemical and biological properties where several common groups of bacteria are especially important to ensure the health of the soil (Egger, 2010). The numbers and species of microbes in soil is depended on environmental conditions like nutrient availability, soil texture, presence of moisture in soil and type of vegetation cover, and other environmental conditions (Brakstad *et. al.* 2015). Bacteria are the largest group of soil microbes, both in total number and in diversity. Bacteria are the only life-forms capable of the biological fixation of nitrogen (Heritage *et. al.*, 1999). In this present research work bacterial strains were isolated from dye effluent and bacterial diversity of contaminated soil samples were characterized on the basis of biochemical and morphological examinations of the obtained colonies depending upon their shape, size, color, color of background, pigment production and types of colony etc. The polluted soil may contain several types of bacterial species. They were identified by Biochemical test includes Indole, Methyl red, Voges-Proskauer test, Citrate, Catalase and gram staining technique. Three Bacterial isolates such as *Bacillus subtilis*, *Bacillus cereus* and *Pseudomonas aeruginosa* were isolated and identified in contaminated soil of Kaithun region and effluent treated soil sample from Pot experiment. Sarnaik *et. al.*, (1995) in a study of soil samples from dye house highlighted low crop yield from the soil irrigated with effluent, owing to the presence of excess soluble salts effluent that is percolated and absorbed by the soil. Also, reduced number of

Discussion

Pseudomonas was reported. The results obtained in this study are in agreement with the Sarnaik *et. al.*, (1995) as *Bacillus* species dominate in the soil. Present results revealed presence of enzyme catalase and citrate in bacterial isolates. Our finding supports begum *et. al* (2017) who reported some bacteria isolated from waste dumping sites in Dhaka city for the presence of enzymes such as protease, oxidase, catalase, coagulase.

Biochemical characteristics of the soil bacterial isolates were studied using different tests like Indole, Methyl red and Voges Proskauer test, Citrate utilization, Urease test, Catalase test, Nitrate reduction test, production of Hydrogen Sulphide as described by Aneja, (1996) for *soil samples*. It was clearly observed that Indole was not produced after incubation of isolated bacterial inoculants in tryptophan broth. Similarly Methyl red and Voges-Proskauer reaction were examined in glucose phosphate broth by adding methyl red and α -naphthol solution with KOH respectively report negative results. Citrate was utilized as a carbon source in Simon's citrate medium and represent as color change for isolated *bacteria i.e.* *Pseudomonas aeruginosa*. Catalase activity was observed by stirring the culture in a drop of hydrogen peroxide (10% by W/V). All the three isolated bacterium showed positive test for Catalase activity. Indra Gandhi *et. al.* (2014) worked on textile effluent and dye contaminated soil and isolated *Alcaligenes spp*, *Bacillus subtilis*, *B.pumilus*, *B.cereus*, *B.megaterium*, *B.licheniformis*, *B.alvei*, *B.macerans*, *B.maxima*, *E.aerogens*, *E.coli*, *Klebsiella pneumoniae*, *Micrococcus spp*, *Lactobacillus spp*, *Pseudomonas florescence*, *P.putida*, *Streptococcus spp*, *Staphylococcus spp*, *S. aureus* and *Serratia spp* and identified by using staining and biochemical test. Our findings similar to the (Arun Prasad and Bhaskara Rao 2010) dye decolorizing isolates, *Bacillus* sp., *Klebsiella* sp., *Salmonella* sp., and *Pseudomonas* sp. were isolated from the textile effluent samples collected from Elampillai, Tamil Nadu. Banerjee *et. al.* (2017) demonstrated that *B. cereus* IB311 has increased the production (20% and 26% in term of average pod number per plant, average seed number per pod, and seed yield per experimental plot) in ground nut (*Arachis hypogaea* var. Koushal, G201) and sesame (*Sesamum indicum* var. Kanak), respectively. They believed that the application of this strain in agricultural field as biocontrolling agent will definitely enhance the production yield and will reduce the disease risk.

(4-E) Isolation and identification of *Rhizobial* strains

As discussed earlier, each legume is nodulated by different species of rhizobium. It is concluded that *Glycine max L.* is nodulated by *Rhizobium japonicum*, and *Medicago sativa L.* is nodulated by *Rhizobium meliloti L.* (*Sinorhizobium meliloti*). Different biochemical tests were proved as valid tests in identification of the organisms. Apart from it some diagnostic features of rhizobium could be conveniently used not only to determine and identify the organism but also specify different species. In the present investigation a comparative study was done between plants grown at control and plants treated with 20% effluent concentration (T₁ treatment level). Comparative study includes isolation, culture and various microbiological tests.

For the study of impact of dyeing effluent on *Rhizobium* bacteria, selected plants were grown in control soil and 20% effluent treated soil, nodule were collected, sterilized and rhizobium were inoculated on YEMA agar. These isolated bacteria from (control and 20% effluent treated) both the legumes were identified by their staining, morphology and cultural characters, all the results were similar to the standard results given by rhizobium as studied by Kumar *et. al.*, (2014), Menna *et. al.*, (2006), Batzli *et. al.*, (1992), Mahana, (1981) Gauri, *et al.*, (2011). Staining and morphology characters were examined from 48-72 hours old cultures, isolated from *Medicago sativa L* and *Glycine max L*, grown in control and 20% effluent treated soils and were examined through gram reaction, after staining Gram negative, pink color short rods were observed, these rods were also arranged in single, in pairs or in clusters. So the rhizobium bacteria are aerobic, non spore forming and motile rods, Staining and morphology characters were examined from 48-72 hours old cultures, isolated from *Medicago sativa L.* grown in control and 20% effluent treated soils and were examined through gram reaction, after staining Gram negative, pink color short rods were observed, these rods were arranged in single, in pairs or in clusters. So the rhizobium bacteria are aerobic, non spore forming and motile rods. Similar morphological, physiological and biochemical characters of rhizobial isolates have been reported by Lalitha and Immanuel (2013) and Rasool *et. al.*, (2015). Pawar *et. al.*, (2014) reported that nodulating bacteria isolated from soybean root nodules showed good growth at temperature 36°C and pH 7.0. 5-10 days old culture grown on YEMA plate examined for colony

Discussion

characters, colonies of *Glycine max*, were circular, convex, whitish pink and glistening with entire margin. These are slow growing bacteria having more than 12 hr generation time. The colony were not exceed more than 1mm in diameter in 5-7days incubation on YEMA. Three days old culture grown on YEMA plate examined for colony characters, colonies of *Medicago sativa L* were circular, convex, whitish pink and glistening with entire margin. These findings are in line with Hussain et. al., (2002), Oblisami (1995) who also isolated the *Sinorhizobium meliloti* from alfalfa with same characteristics.

The bacterium showed well-marked growth on YEMA medium at PH 7.0. Mahana, *et al.*, (2000), reported that the Rhizobium isolated from *Vigna mungo L*. showed marked variations in growth with respect to time period on YEMA while they do not show any growth on Hofer's alkaline medium at pH 10.0 with slight growth and evolution of gas and acid production. The pattern of growth in the motility agar stab culture of rhizobium of *Glycine max L* was observed after 48-72 hr of incubation, and observed that bacteria move slowly from the stab line in to medium. Rhizobium of *Medicago sativa L* move slowly but comparative faster than rhizobium of *Glycine max*. L. (Aneja, 2008; Basak and Goyal, 1980).

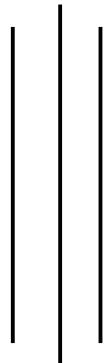
Biochemical characteristics of the Rhizobium isolates were studied using different tests like Indole, Methyl red and Voges Proskauer test, Citrate utilization, Urease test, Catalase test as described by Aneja, (1996) for *Medicago sativa L* and *Glycine max L*. It was clearly observed that Indole was not produced after incubation of isolated rhizobial inoculants in tryptophan broth. Similarly Methyl red and Voges-Proskauer reaction were examined in glucose phosphate broth by adding methyl red and α -naphthol solution with KOH respectively report negative results. Citrate was utilized as a carbon source in Simon's citrate medium and represent as color change. Catalase activity was observed by stirring the culture in a drop of hydrogen peroxide (10% by W/V). The bacterium showed positive test for citrate and Catalase activity. Mahana, *et. al.*, (2000) and Maheshwari *et. al.*, (2012) reported Catalase activity in some isolates from *Vigna mungo* and *E.coli*. The bacterium is negative for MR-VP and Indole reaction reported by Gachande and Khansole, (2011) Similarly, Graham and Parker, (1964), did not observe MR reduction in all the isolates of seven rhizobia groups. While Basak and Goyal, (1980), also reported that none of the rhizobial isolates of seven groups produces

Discussion

Indole. The isolates under study were confirmed as slow growing *Bradyrhizobium* since they showed an alkaline reaction turning the growth medium blue. *Rhizobium japonicum* syn. *Bradyrhizobium- japonicum* is associated with the root nodules of Soybean and fixes 100 kg nitrogen/ha/year, (Purohit and Kumar, 1998). Deka and Azad, (2006) studied the rhizobial isolates from 6 common pulses justify our results. Our these findings are in close agreement with Elsheikh and wood (1989); Javed and Asghari (2008) who also reported characterized the rhizobium from soil and sunflower root nodules with the same positive biochemical tests. The positive samples were also subjected to sugar fermentation tests and were found positive to glucose confirming the bacterial species. These findings verified with the results of oblisami (1995); Michael (2006); Singh (2008) and Erum (2008) who also reported this sugar test positive during isolation and characterization of *Rhizobium meliloti*.

Shahzad *et al.* (2012) isolated *Rhizobium* from root nodules of Alfalfa (*Medico sativa*) plant and characterized on the basis of various biochemical tests. Previously, Sadowsky *et al.* (1983) mentioned that fast-growing soybean rhizobia were positive for catalase, urease, oxidase, nitrate reductase, tolerated 2% NaCl, capable to grow at pH 9.5 and fermented L-arabinose, D-fructose, D-galactose, D-glucose, D-mannitol, D-mannose, L-rhamnose and D-xylose. Similarly, Singh *et al.* (2008) also characterized *Rhizobium* strains on the basis of biochemical tests. *Rhizobium* is symbiotic bacteria which form nodule in leguminous plant.

CHAPTER – 6



CONCLUSION

CONCLUSION

In the present research work it has been concluded that the textile and printing industries play an important role in the economic growth of India but on the other hand dyeing effluent is considered as one of the most environmentally unfriendly industrial waste. This study has shown that dyeing and printing clusters of Kaithun region, Kota are utilizing a huge amount of water at various stages of dyeing and printing process. A number of Azo dyes were used in dyeing and printing industries. Untreated wastewater was being discharged on the open field or in the nearby water bodies, was chosen for this study. The waste water is carried to the main drainage system as a result the water quality becomes turbid with blackish brown color which is detectable over long distances. The contaminated water is utilized for irrigation in agricultural fields by the local farmers. Besides this, effluent of these printing units also results in soil and land pollution due to disposal of fabric swatches, scraps and gums used in printing. The residue of fabric swatches are also piled up in front of the industry. The swatches since cannot be sold or weight are thrown on the streets which produces enormous odour also resulting in blockage of drains during rainy season. In the present investigation, an attempt has been made to assess the physico-chemical characteristic of effluent and soil and to evaluate the effects of dyeing and printing effluent on seed germination, seedling growth, yield, biochemical parameters and nodulation. Kaithun region is well known for using dyes for dyeing and hand block printing. The results showed that effluent is slightly alkaline (pH- 7.9), foul smelling and highly colored. Other parameters like total hardness, TDS, BOD and COD values exceed the permissible limits at significant level. The concentration of heavy metals was very close to the permissible limit which might be due to the use of mordents and synthetic dyes. The inorganic insoluble salts as Ca and Mg makes water hard and unsuitable for living organisms. The textile dyeing effluent is highly polluted to the environment as physicochemical parameters of dye effluent including pH, COD, BOD and TDS exceeded the BIS- standard permissible limits. Therefore, the effluent of the dyeing and printing industries of Kaithun region is unsuitable for the existence of biodiversity in the environment.

Conclusion

The results indicate that the discharge of untreated effluent affect physico-chemical properties of soil. Results revealed that soil is slightly alkaline (pH- 7.9-8.1), % organic carbon, sodium, phosphorus and potassium content was high and are not in compliance with standards. Therefore, the dyeing and printing effluent should be treated before dumping to open place or in water bodies. From the results it can be concluded that seed germination, seedling growth, yield, biochemical parameters and nodulation negatively affected when treated with dyeing and printing effluent. The effect of dyeing effluent on seed germination, seedling growth, yield, nodulation and biochemical parameters of *Glycine max* L. and *Medicago sativa* L. was analyzed in the present investigation. In this study control, 20% (T_1), 40% (T_2), 60% (T_3), 80% (T_4) and 100% (T_5) effluent was used as treatment levels. Seed germination and seedling growth of both the legumes was enhanced at T_1 (20%) treatment level increasingly as compared to the control. With the increasing concentration of effluent seed germination, seedling growth, biomass, pigment content, free sugar, protein, amino acid content and other parameters were reduced or negatively affected. It can be concluded that dyeing effluent as such inhibit the growth whereas with the dilution it promotes the germination and growth parameters of *Glycine max* L. and *Medicago sativa* L. Concentrated dyeing effluent was heavily loaded with pollutants which negatively affect plant growth and yield by interfering with nutrient uptake and physiological process. However, on dilution toxic effects of the effluent were reduced and its effects on growth, yield, physiological and biochemical parameters could be stimulatory rather inhibitory. The untreated dyeing industry effluent could possibly lead to soil deterioration and low productivity. In conclusion, dyeing industry effluent at various concentrations influences seed germination and seedling growth of *Glycine max* L. and *Medicago sativa* L. However the effects vary from crop to crop because each plant species has its own tolerance of the different effluent concentrations. From the interview of local farmers it was found that there was a great problem to the environment due to these dyeing and printing units and crop productivity was also negatively affected due to irrigation practice by effluent/wastewater.

Conclusion

During the present research work bacteria like *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Bacillus cereus* were isolated from the soil polluted by dyeing and printing effluent. As Bacteria's is a tiny and lower most component of any food chain. But these tiny members have their own importance, without bacteria we can't imagine any food chain. But these important members can survive in good quality of soil, in place of it they return fertility of soil by maintaining NPK contain of soil.

This study is helpful to analyze the impact of dyeing effluent on leguminous plants and to study not only the morphological aspect but also the biochemical & microbiological aspect of the root nodule bacteria of leguminous plants. Result indicates some negative impact of higher concentration of dyeing and printing effluent on which we can do further research to minimize the environmental hazards and some safety measures can be suggested. Data generated in the present study may be useful to understand the positive impact on growth performance of *Glycine max L* and *Medicago sativa L* treated with dyeing effluent that can be used as fertilizer after some alteration, and used in agronomy. Our ultimate effort is to study the impact of dyeing effluent on root nodules and study of microbiology and biochemical analysis of selected legume by isolating inoculating and culturing the rhizobium bacteria. In the light of above facts it is concluded that dyeing effluent up to 20% doesn't affect the rhizobium bacterial growth as well as its characters, so treated effluent can be used in future for soil amendments in different ways . Rhizobial strain viz. *Rhizobium japonicum* and *Rhizobium meliloti* can enhance the nitrogen fixation leads to improve soil quality so floristic diversity of area will remain rich always. The wastes of dyeing and printing industries primarily contain organic wastes and are not always harmful when properly used. The effluents of various industries are generally rich in essential plant nutrients and organic matter but sometimes have high concentration of metals. Thus, the effluent should be properly treated before their use in irrigation (Hoddy, 1991; Sammy *et al*; 1995).

Hence, the treated dyeing industry effluent may be suitable for fields with acidic soils and it not only solve the disposal problem but also serves as an additional source of fertilizer in liquid form. This preliminary study proves the

toxicity of dyeing effluent on both the legumes. A further study is needed to confirm the effluent toxicity with more analyses. Our study surely helpful in the direction of treated effluent utilization and enlightens the way to future research which must be beneficial for maintaining the biodiversity of the area.

Future Prospects:

Dyeing effluents are not only toxic to the aquatic ecosystem but also carcinogenic for human beings and once they enter into the water system, posses potential risk to the life. These effluents having toxic dyes and heavy metals may have negative effect on soil and plants. Irrigation done with untreated effluent may cause phytotoxicity and entry of pollutants into the food chain. The results indicated that the untreated dyeing and printing effluent used for a long periods in soils cause deleterious effect on physico-chemical properties of soil. The study suggests that continues application of effluent deteriorate soil quality and soil fertility. There is an urgent need for proper management practices of waste water/polluted water for irrigation purpose.

In the present investigation it has been concluded that the irrigation with 20% treated effluent was the best for this purpose and could full fill the fertilizers requirements of crops. Field investigation is needed to confirm its potential on legumes/crop plants under natural soil environment. Diluted effluent has shown better growth and yield of plants as utilization of nutrients from the effluent thus reducing the requirements of artificial fertilizers with systematic wastewater management.

There is a need to study the variation and evolution of microbial species which naturally treat the dye effluent, i.e., dyes and dye residues. Diverse species of microbes can utilize dye compounds as their carbon source and energy can be selected to treat industrial effluent.

The present study can be significantly helpful to understand the diversity of rhizobia in regional crops and associated with the studied plant. Due to safe, cost

Conclusion

effective and positive impact on agricultural field, this bacterial strain will be a good bio-controlling agent.

- There should be a common effluent treatment plant (CETP) to be operated solely by Government of Rajasthan or it should be operated jointly by Industry owners and Government.
- Untreated effluent must not be allowed to be discharge in to drain system or open filed.
- Industries may be encourage through extending subsides for utilizing natural dyes with processed and bio mordants.
- While using the effluent from the dyeing and printing industry for the irrigation purpose, proper dilution of the effluent is a must for the low negative impact.
- Further investigations on assessment of toxicity of effluent, bioaccumulation of heavy metals in crops and its impact on human being should be done.
- Awareness programme should be launch to the local farmers about the impact of effluent and its proper utilization.

CHAPTER – 7



SUMMARY

SUMMARY

“Physico-chemical Study of Dye effluent and Its Impact on Soil and Nodulation in some Leguminous plants”

Chapter 1: Introduction

Industrialization is the most important factor for economic development of a country, since it provides huge employment and is a mean of foreign exchange. But at the same time, it is the major cause of water and environmental deterioration. Rapid development of different industries like chemical, food processing, metal working, pulp and paper, petrochemical, refining and textile industries, both in developed and developing nations in last few decades have resulted in generation of huge amounts of wastewater that is released on land and into various water bodies. Textile dyeing and printing industry is one of the major water consuming and highly polluting industries in India. The industrial pollutants or effluent caused alteration in physicochemical and biological properties of the environment. Textile wastewater has a highly diverse composition containing a large number of organic and inorganic compounds like acids, dyeing bases, caustic soda, sodium hydrosulphate, mordents, reducing agents soap and heavy metals, dyestuff, optical bleachers, finishing chemicals etc. (Spagni *et al.*, 2012). The presence of dye color in water bodies is not only an aesthetic problem, but also prevents sunlight penetration through the water surface, thus disturbing the ecosystem (Joshi *et al.*, 2011). The textile dyes and effluent have toxicant effects on the germination rates and biomass of many plant species that have vital ecological functions, like providing a surroundings for life, protective thus from pollution and providing the organic matter that's so important to soil fertility (Kapustka and Reporter, 1993). Dyeing and printing effluents from various cluster units were discharged on open land and agricultural land thus causing an adverse effect on flora, fauna and general health of residents.

Kaithun district is called as the textile (Dyeing and printing) city. It is covered by many small scale textile dyeing and printing units. It plays an important role in creating water pollution by discharging effluent directly into nearby water bodies and on land. Indiscriminate use of industrial effluent may cause pollution problems in the long run when are not properly handled before and after their application to irrigated land. With this background a study was taken up to investigate the physico-chemical analysis of effluent generated by textile dyeing and printing mills for irrigation purpose and its impact on soil and nodulation in some leguminous plants. *Rhizobia* are one of the most efficient bacterial symbionts of legumes that fix atmospheric nitrogen by the process of biological nitrogen fixation. Keeping in view the importance of *Rhizobia* in legume plants, the present study was undertaken to shed some light on different morpho-physiological and biochemical properties of Rhizobial strain isolated from *Glycine max* L. and *Medicago sativa* L.

In this present study an attempt has been made to identify the effect of textile dye effluent on seeds germination, seedling growth, yield, biochemical characteristics and nodulation study (Microbial study) of leguminous plants.

Chapter 2: Review of literature

Effluents discharged from the industries have either beneficial or lethal effects on germination, growth and development of agricultural crops. The waste water from the dyeing and printing units used for irrigational purposes in agricultural land that can cause various damages to the plants and soil and reduce the soil fertility due to the toxic nature of the effluent. Chhonkar *et. al.*, (2000) characterized effluent originate from textile industry, Pali (Rajasthan) with high salinity (SAR: 82), BOD (400-800 mg L⁻¹), COD (900-1500 mg L⁻¹), excessive concentrations of sodium and carbonate ions (RAS: 42 m eq L⁻¹), high alkalinity (pH: 10-11.5) and unduly low concentrations of calcium. Kathirvel (2012) studied the effect of dye factory effluent with respect to germination and growth of Bengal

Summary

gram *Cicer arietinum* L. He observed that lower concentration of dye effluent, the germination percentage and growth are relatively higher than the control, but with the increasing in effluent concentration the gradual decrease in the germination of seeds, seedling growth was observed. The best germination, seedling growth, number of root nodules, yield was observed in 20% concentration with growth promoting effect and significantly better than control. Beyond 20% effluent, root and shoot length decreased. Ramya *et. al.*, (2017) studied on the effect of effluent with respect to germination, morphology and biochemical characters of *Arachis hypogaea* L. var. K6. Dilution effluents (25%, 50%, 75% and 100%) and treated effluent were irrigated on *Arachis hypogaea* L. Seed germination and seedling growth were gradually decreased with increase in effluent concentrations but the best seedling germination and growth was observed in treated effluent compared to other doses. All concentrations of textile effluent were injurious and reduction on plant growth and its morphological parameters of hypocotyls length and epicotyls length. Number of root branches was observed in all effluent concentrations and mostly root affected by 100% textile effluent compared with control. Biochemical characters of amino acids and protein were also reduced with the increasing effluent concentrations. Morphological and biochemical characters show better growth in treated effluent and followed by 25% effluent than all other concentrations of effluent. Brown and Wilkins (1986) and Dayama (1987) studied the influence of dyeing and textile waste water on nodulation and germination of *Cicer aeritium*. Srivastava and Sahai (1987) studied the impact of various concentrations of distillery effluents (1, 2.5, 5, 10, 25, 50, 75 and 100 %) on seed germination, speed of germination, germination index, growth, leaf area, biomass, net primary productivity, reproduction capacity, seed output, seed weight, seed densities content of *Cicer arietinum* and reported up to 5% concentration was beneficial for the overall growth parameters and its use as a liquid fertilizer has been suggested. Gowsalya *et. al.*, (2014) stated that soil contains many types of microorganisms such as bacteria, actinomycetes, fungi, algae and protozoa which are important because they affect the all (Physical, chemical and biological) properties of soil.

Among the soil bacteria a unique group called *Rhizobia* has a beneficial effect on the growth of plants. It can live either in the soil or within the root nodules of host legumes. Legume plant posses a unique ability to establish symbiosis with nitrogen fixing bacteria of the family *Rhizobiaceae*. Komy, (2005) concluded that Legumes have been suggested as appropriate crops for the enhancement of bioproductivity and reclamation of marginal lands, because these plants not only yield good fodder, protein rich seeds and fruits, but they also enrich soil nitrogen in symbiotic association with *Rhizobium*. Junior *et. al.*, (2005) reported that Legume plant may utilize wastewater effluent and uptake heavy metals through extensive root system. Thus, these plants serve as effective biological sieves, inhibiting contamination of ground water sources. Goormachtig *et. al.*, (2004) concluded that nodulation is adversely affected by salinity, which can adversely affect legume growth or reduce crop yield. Junior *et. al.*, (2005) stated that nodulation and nitrogen fixation in *legume- Rhizobium* association are adversely affected by water quality can reduce plant growth and crop yield.

Hence, reuse of dyeing effluent or waste water in agriculture is an alternative strategy of eco-friendly disposal, which increase and improving the productivity of crops and plants. Since, the literature pertaining to recycling of dyeing and printing effluent is very limited, so the present investigation entitled “**Physico-chemical study of Dye effluent and Its Impact on Soil and Nodulation in some Leguminous plants (i.e. *Glycine max L.* and *Medicago sativa L.*)** was carried out on the dyeing and printing effluent discharging from small textile units from Kaithun region, Kota, Rajasthan.

Chapter 3: Material and Methods

Physico-chemical analysis:

Effluent and contaminated soil were analyzed by using APHA method (1998).

Preparation of various treatment level of effluent

Sample
C-control (Tap Water)
T1-plants treated in 20% effluent + 80% Distilled water
T2-plants treated in 40% effluent + 60% Distilled water
T3-plants treated in 60% effluent + 40% Distilled water
T4-plants treated in 80% effluent + 20% Distilled water
T5-plants treated in 100% raw effluent

Experimental Plants:

Glycine max L (JS 335) also known as Soya bean. The bean pods and seeds are a source of oil and protein and are good source of phytic acid, dietary minerals and vitamin B. Traditional unfermented food uses of soybeans include soy milk, from which tofu and tofu skin are made. Fermented pods are used to make soya sauce and other sauces and soya milk. Inoculation with nitrogen-fixing bacteria is desirable, the strain *Rhizobium japonicum* being specific to soya bean.

Medicago sativa L (T9) also known as alfalfa It is one of the highest yielding forage legumes. It is grow as a cover crop to reduce erosion. It is a nitrogen fixer. It has medicinal properties and a yellow dye and trypsin inhibitors can be extracted from the seeds. They are typically high in vitamin K and also contain vitamin C, copper, manganese and folate and they are also very low in calories. It is compatible with non-aggressive grasses. It is inoculated with an effective strain of *Rhizobium meliloti*.

Germination study:

Germination study by using Petri plate method.

Growth and Biochemical analysis: By Pot Experiment

I. Morphological parameters :

Shoot length and Root length measured by Centimeter scale, Shoot and Root fresh weight, Shoot and Root dry weight by Buris *et al.*, method (1969), Vigour index by Abdul Baki and Anderson method (1973), Nitrogen, Phosphorous, Potassium content in shoots and roots by Micro-kjeldahl method (1883).

II. Pigment content :

Chlorophyll a, Chlorophyll b, Total Chlorophyll, Carotenoids by Arnon's method (1949).

III. Biochemical contents:

Total protein by Lowry *et al.* (1951), Free amino acids by Moore and Stein, (1948), Free sugar by Nelson, (1944).

IV. Yield productivity:

The yield productivity (Pods per Plant, Pods weight, Seeds per pod, Seeds weight) were determined by using counting and weighing machine at completion of life cycle of both experimental plants i.e. *Glycine max L.* and *Medicago sativa L.*

V. Nodulation study:

The nodulation study includes Nodules per plant, Nodules shape, Nodule color by simple observation and Fresh weight of nodules, Dry weight of nodules were measured by using weighing machine, Lag hemoglobin content was measured by Sadasivam and Mannickam (1992) method at 30 DAS and after completion of life cycle of both experimental plants i.e. *Glycine max L.* and *Medicago sativa L.*

VI. Microbial Analysis

(A) Soil Microbial study:

Isolation and identification of soil bacterial species from Pot experiment were done by using serial dilution and some biochemical test such as Indole test, Methyl red test, Voges-Proskauer test, Citrate utilization test, Catalase test given by Aneja (1998).

(B) Rhizobium Isolation:

The morphological and biochemical characteristics of authenticated Rhizobial isolates were studied following the Indole test, Methyl red test, Voges-Proskauer test, Citrate utilization test, Catalase test given by Aneja (1998). In order to test the significance of data, the statistical analysis of recorded data was made with standard procedures. The method of standard deviation is used to find out the deviation between various parameters of present study. In order to establish interrelationship between various parameters graphical presentations were made which are presented in thesis.

Chapter 4: Results and Discussion

(A) Physico-chemical analysis:

In this present research work physicochemical analysis of dyeing and printing effluent and contaminated soil have been done using APHA (1998). Result showed that pH ranged from 7.20-7.97 in effluent samples S_1 and S_2 . Electrical conductivity ranged from 1470-2200 .Color was dark blue and black in both samples. Biological oxygen demand (488-1090 mg/l), Chemical oxygen demand (3120-6864 mg/l), Calcium hardness (110-800 mg/l) , Magnesium hardness (120-1450 mg/l), Total hardness (230-2250 mg/l), Total dissolved solids (980-1440 mg/l), Oil and grease (21-43.6),Heavy metals such as Zn, Cu , Mn and Fe were also found in S_1 and S_2 within permissible limit by BIS For irrigation. Similarly physicochemical analysis results for soil samples S_1 , S_2 and S_3 showed pH ranged

from 7.99-8, Electrical conductivity was found 0.21-0.44, Organic carbon (%) ranged from 0.53-0.95. Sodium (256-622), potassium (16.6-49.9), potash (314-653), calcium (2.9-3.6), magnesium (3.4-4.0), % water holding capacity (37.91-45.86), Zn (0.339-6.886), Cu (0.887-48.65), Mn (4.318-11.50) and Fe (0.253-9.02) higher than permissible limit. Kumar *et al.*(2013) investigated Physico-chemical and heavy metals characteristics of bore well water and textile effluent in Haridwar. For this purpose irrigation water were collected and analyzed for various parameters *viz.*, TDS, EC, pH, BOD, COD, Cl⁻, K⁺, Na⁺, Ca²⁺, Mg²⁺, NO³⁻, PO³⁻, SO²⁻ and Fe²⁺ along with heavy metals. Among various parameters, BOD, COD, Cl⁻, Ca²⁺, NO³⁻, SO²⁻, Fe²⁺ and heavy metals content were found beyond prescribed limit of Indian standards.

(B) Morphological, Pigment and Biochemical Parameters:

Germination percentage, morphological parameters (Shoot and Root length, fresh weight and dry weight of Shoot and Root, Vigour index) were increased at treatment level T₁ (20%) and T₂ (40%) in comparison to control while decreasing at T₃ (60%), T₄ (80%), T₅ (100%) treatment level in comparison to control at 30 DAS and completion life cycle of both the leguminous experimental plants i.e. *Glycine max* L. and *Medicago sativa* L. The Pigment content (Chlorophyll a, Chlorophyll b, total Chlorophyll and carotenoids) of both plants showed a gradual decline with the increasing concentration of effluent. Significant loss of pigment concentration occurred in the plants those were treated with 60%, 80% and 100% treatment levels Whereas at T₁ and T₂ pigment content was higher than control. Dilution of effluent positively affects the plant growth and pigment content. From the data it became evident that pigment content was maximum at T₁ (20%) treatment level than all other treatments including control. Biochemical parameters were also estimated at 30 DAS and after completion of life cycle of both the experimental plants. Free sugar, protein and amino acid were increased with the T₁ (20%) dilution of effluent. With the increasing concentration of effluent these parameters were negatively affected. The productivity of *Glycine max* L. and *Medicago sativa* L. plants negatively affected with the higher concentration of effluent. Due to deteriorating

effects of effluent on roots the nodule characters were also negatively affected. Narain et. al., (2012) also stated that the distillery effluent did not show any inhibitory effect on seed germination, germination index, plumule and radicle length, fresh and dry weight of plumule and radicle, chlorophyll and carotenoid contents of *Cicer arietinum* at low concentration (25%). The germination percentage of seed, seedling growth and chlorophyll contents showed a gradual decline with the increase in the effluent concentration which also supporting our finding. Similarly Medhi (2011) also studied the effect of dye factory effluent at 20% dilution and reported that yield characters are relatively higher than the control, but gradually decrease with the increase in effluent concentration.

(C) Soil microbial analysis:

Some bacterial species were isolated and characterized by biochemical testing (iMVic test) i.e. *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Bcillus cereus*. These bacterial strains are identified in the micro-diversity of textile effluents contaminated soil and can be studied for symbiosis relationship with rhizobial strains of leguminous plants.

(D) Rhizobial strains analysis:

At T₁ (20%) treatment level rhizobial species characterized i.e. *Rhizobium Japonicum* which shows symbiotic relationship with soil bacteria i.e. *Pseudomonas aeruginosa*, *Bacillus subtilis* and increase the nodules productivity while in control only *Rhizobium Japonicum* and *bacillus substilis* were found in *Glycine max L.* (Soybean- JS 335). In T₁ (20%) treatment level bacterial species characterized i.e. *Rhizobium meliloti* , shows symbiotic relationship with soil bacteria *Bcillus cereus* and *Bacillus subtilis* while in control *Rhizobium meliloti* and *bacillus substilis* were found in *Medicago sativa L.* (Alfalfa- T9). Goel and Mandavekar (1983) at Karad (India) observed tilt cluster bean (*Cyamopsis letragonoloba L.*) if irrigated with 10% distillery wastewater recorded more nodulation. However, the higher concentration of distillery waste increased the salt content and organic matter in the

soil resulting into the suppression of nodulation. Our research findings are supported by these workers as Graham and Parker, (1964) Vincent, (1970), Gaur, (1975), Mahana, (1981), Mahana, *et al.*, (2000) found same result when studied various legume.

Chapter 5: Conclusion

Dyeing industrial effluent significantly influence growth parameters of the plants due to their toxic nature. The result suggested that diluted dyeing and printing effluent (less than 20%) could be used for irrigation. Phytoremediation may contribute in the treatment of various sites contaminated with industrial effluent. Hence, this preliminary study proves the toxicity of untreated dyeing industry effluent on *Glycine max* L. and *Medicago sativa* L. The presence of *Rhizobial* strains in root nodules of the plants. Higher concentration of dyeing effluent also affects the morphology and biochemistry of nodules. The presence of rhizobia in root nodules enhances the soil fertility and agricultural production. These findings can be significantly helpful to understand the diversity of *Rhizobia* and allow us a new scope for extensive research in Agriculture Biotechnology. A further study is needed to confirm the effluent toxicity with more analyses.

CHAPTER – 8



BIBLIOGRAPHY

BIBLIOGRAPHY

1. Abdul Baki A.A., and J.D., Anderson, (1973). Vigour determination of soybean seeds by multiply criteria. *Crop, sci.*, 13: 630-633.
2. Ahmad, M. T., S., Manderia, and K., Manderia (2012). Influence of dye industrial effluent on physico chemical characteristics properties of soil at Bhairavgarh, Ujjain, MP, India. *International Research Journal of Environmental Science*, (1): 50-53.
3. Ajmal, M. and A. U., Khan 1985. Effect of a textile factory effluent on soil and crop plants. *Environ. Pollut.*, 37: 131–148.
4. Albino Wins, J. and M., Murugan (2010). Effect of textile mill effluent on growth and germination of black gram – *Vigna mungo* (L.) Hepper. *International Journal of Pharma and Bio sciences*, 1: 1-7.
5. Al Jasser A.O. (2011). Saudi wastewater reuse standards for agricultural irrigation: Riyadh treatment plants effluent compliance. *Journal of King Saud University, Engineering Sciences*, 23: 1-8.
6. Alloway, B. J., (1990). Heavy metals in soils. *John Wiley and Sons*, Inc New York.
7. Aneja, K.R., (1996). Experiments for microbiology, plant pathology and tissue culture. *Wishwa prakashan*. New Age International (P) Limited, New Delhi, pp. 190-217.
8. Aneja, K.R., (2008). Experiments in microbiology, plant pathology and biotechnology. Fourth Edition, *New Age International Publishers Limited*.
9. Anoop Singh, S B., Agrawal, JPN., Rai and P., Singh (2002). Assessment of the pulp and paper mill effluent on growth, yield and nutrient quality of wheat (*Triticum aestivum* L.) *J. Environ. Biol.*, 23(3): 283-288.

Bibliography

10. Aoyama, M. and S. Kuroyanagi, 1997. Effects of heavy metal accumulation associated with pesticide application on the decomposition of cellulose and orchard grass in soils. *Soil Sci. Plant Nutr.*, 42: 121-131.
11. APHA (American Public Health Association), (1998). Standard methods for the examination of water and wastewater (20th ed.), Washington, DC.
12. APHA (2012). Standard methods for the examination of water and wastewater. 21th Edn., APHA, AWWA, WPCF, Washington D.C.USA.
13. APHA (American Public Health Association) (2012). Standard Methods for the Examination of Water and Wastewater, 22nd Edn., 45-60.
14. Arjun, K. S., S. Kumar, Y. Kumar and H.C., Sharma, (2013). Effect of fertilizer factory effluent on wheat Crop: A case study. *Access International journal*, 1(7): 81-90.
15. Arnon, D.I. Copper enzymes in isolated chloroplasts (1949). Polyphenoloxidase in Beta vulgaris. *Plant Physiol.*, 24: 1-15.
16. Arora, S., P., Ranjan, G. S., Sharma, J. S., Sindhu, G., Singh, A. K., Singh and V. K., Kansal (2005). Effect of calcium fortification on heat stability and physico-chemical properties of mixed (cow and buffalo 1:1) milk. *Indian Journal of Dairy Science*, 58(4): 242-246.
17. Arora, S., A. K., Chopra, G., Prasad, N., Joshi and G., Prasad (2006). Characteristics of Mahalakshmi sugar mill effluent and its impact on seed germination on certain agricultural crops. *Journal of Applied Biosciences*, 32: 15-118.
18. Arun Prasad A.S., and K.V., Bhaskara Rao (2010). “Physico-chemical characterization of textile effluent and screening for dye decolorizing bacteria”. *Global Journal of Biotechnology and Biochemistry*, 5: 80-86.

Bibliography

19. Arya, M. P. S. and R., Singh (1996). Effect of N, P and K, on the nodulation, growth and yield characters of horse gram [*Macrotyloma uniflorum* (Lam) verdc.]. *Legume Research*, 19: 65-69.
20. Aswathi, K., M. R., Rajan, and D. Noel. S. (2013). Impact of Dyeing Industry Residue on Growth, Biochemical Characteristics and Yield of Bhendi *Abelmoschus Esculentus*. *Ind. Jour.of Appl. Res.*, 3(11): 220-222.
21. Baath, E. 1989. Effects of heavy metals in soil on microbial processes and populations (a review). *Water Air Soil Pollut.*, 47: 335-379.
22. Bahadur and B.K., Sharma (1990). Effect of Industrial Effluent on Seed Germination and Early Seedling Growth of *Triticum aestivum* Var. UP 115. *Acta. Bota. Indica.*,18: 80-83.
23. Balakrishnan, M., S., Arul Antony, S., Gunasekaran, and R.K., Natarajan (2008). Impact of dyeing industrial effluents on the groundwater quality in Kancheepuram (India). *Indian Journal of Science and Technology*, 1: 1-8.
24. Balashouri and Prameela Devi (1994). Growth and physiological activity of green gram under effluent stress. *J. Eco. Environ. Mon.*, pp: 4-115.
25. Banat, I.M., P., Nigam, D., Singh and R., Marchan (1996). Microbial decolorization of textile-dye containing effluents. A review. *Bio resource Technol.*, 58: 217-227.
26. Banerjee, G., S., Gorthi and P., Chattopadhyay (2017). Beneficial effects of bio-controlling agent *Bacillus cereus* IB311 on the agricultural crop production and its biomass optimization through response surface methodology. *Annals of the Brazilian Academy of Sciences*, 90(2): 1-18.
27. Basak, M. K. and S. K., Goyal (1980). Studies on tree legumes 3rd characterization of the symbionts and direct and reciprocal cross inoculation studies with tree legumes and cultivated legumes. *Plant and Soil* 56: 39-51.

Bibliography

28. Basak M. K. and S K. Goyal (1980). Studies on the biology of tree legumes-Rhizobium symbiosis. Nodulation pattern and cross inoculation trials with tree legumes and cultivated legumes. *Annals of Arid Zone.* L9: 427-430.
29. Baskaran, L., P., Sundaramoorthy, A.L.A., Chidambaram and G. K., Sankar (2009). Growth and physiological activity of greengram (*Vigna radiate* L.) under effluent stress. *Botany Research International*, 2 (2): 107-114.
30. Batzli JMC., WR., Graves, PV., Berkum (1992). Diversity among rhizobia effective with *Robinia pseudoacacia*. *Appl Environ Microbiol*, 58: 2137-2143.
31. Begum K., S.J., Mannan , R., Rezwan , Md. M., Rahman, Md. S., Rahman and A., Kamal (2017). Isolation and Characterization of Bacteria with Biochemical and Pharmacological Importance from Soil Samples of Dhaka City, *J. Pharm. Sci.*, 16(1): 129-136.
32. Bharati S., and N. P., Shinkar (2013). Dairy Industry Wastewater Sources, Characteristics & its Effects on Environment. *Int. J. of Current Eng. Technol*, 3(5): 1611-1615.
33. Bhatnagar, A.R., Pathak, K.C., and Dodia, P. 1986. Effect of sugar factory effluent on germination and growth of rice (*Oryza sativa*). *Environ. Ecol.* 4: 218-220.
34. Bhatnagar, M. and S., Gupta (2002). Impact of dairy effluents on physico-chemical characteristics of soil. *Asian Journal of Chemistry*, 14: 300-304.
35. Bishnoi, O.P. and S., Roy (2017). Physico-Chemical Studies of Effluent in Sanganer Area. *International Journal of Pharmaceutical & Biological Archives*, 8 (1): 34-37.
36. Bohn, H.L., B.L., McNeal, A.G.O., Connor (1985). Soil Chemistry, second ed. *Wiley Interscience Publication*, New York, USA.

Bibliography

37. Brakstad, O.G., M., Throne-Holst, R., Netzer, D.M., Stoeckel, and R.M., Atlas (2015). Microbial communities related to biodegradation of dispersed macondo oil at low seawater temperature with Norwegian coastal seawater. *Microb Biotechnol.*, 18: 989-98.
38. Bureau of Indian Standards Drinking Water Specification IS: 10500; 1991.
39. Chander, K. and P.C. Brookes (1991). Effects of heavy metals from past applications of sewage sludge on microbial biomass and organic matter accumulation in a sandy loam and silty loam U.K. soil. *Soil Biol. Biochem.*, 23: 927-932.
40. Chandy, J. P. (1999). Heavy metal tolerance in chromogenic and non-chromogenic marine bacteria from Arabian Gulf. *Environmental Monitoring and Assessment*, 59(3): 321–330.
41. Choudhary, S.K., Jha, A.N. and Srivastava, D.K. 1989. Impact of paper mill effluent on germination, seedling growth and pigment content of Hordeum vulgare. *Environ. Ecol.* 7: 193- 195.
42. Chinnusamy, C., Annadurai, K., Jayanthi, C., Veeraputhiran, R. and Karunanthi, S. 2001. Organic amendments and distillery effluent on soil fertility and productivity of rice. *Proceedings of National seminar on use of poor quality water and sugar industrial effluents in Agriculture*, Feb.5, TNAU, Tiruchirapalli, p: 84.
43. Correia, V.M., T., Stephanson and S.J., Judd (1994). Characterization of textile wastewaters – a review. *Environ Technol.*, 15: 917-929.
44. Cottene (1973). Chemical fertilizer in relation to agriculture productions. *Medelingen Faculteit Land Bouwwe Tenschappen. Genetics.*, 38(4): 1722-1731.
45. CPCB (Central Pollution Control Board) (1990). Minimal national standards: Dye and dye Intermediate industry. *Comprehensive Industry Document Series: COINDS / 34/1990.*

Bibliography

46. CSIRO, (1995). Effluent irrigated plantations: design and management. CSIRO Technical paper No. 2. *Canberra*.
47. Dass D. and R.N., Kaul (1992) Greening wasteland through wastewater. *National Wastelands Development Board, Ministry of Environment and Forest*, New Delhi, India: 33.
48. David, N.S., and M.R., Rajan (2012). Assessment of water quality index of dye industry effluent and its irrigation standard. *Indian Journal of Environmental Protection*, 29 (2): 145-151.
49. Dayama, O.P. (1987). Influence of dyeing and textile water pollution on nodulation and germination of gram. *Acta ecologica*, 9: 34-37.
50. Deepali and K. K., Gangwar (2010). Metals Concentration in Textile and Tannery Effluents Associated Soils and Ground Water. *New York Science Journal*, 3(4): pp 82-89.
51. Deka, A. K. and P., Azad (2006). Isolation of Rhizobial strains cultural and Biocemical Characteristics. *Legume. Res.*, 29(3): 209-212.
52. Deshwal V.K. and A. Chaubey (2014). Isolation and Characterization of *Rhizobium leguminosarum* from Root nodule of *Pisum sativum L.* *Journal of Academia and Industrial Research*, 2(8): 464-467.
53. Dhanam, S. (2009). Effect of dairy effluent on seed germination, seedling growth and biochemical parameters in paddy. *Botany Research International*, 2: 61-63.
54. Divyapriya, S., Dimi., Divakaran, K. P., Deepthi, (2014). Biochemical effect of industrial effluence on germinating seeds of *cicer arietinum*. International Journal of Pharmacy and Pharmaceutical Sciences, 6(2): 538-542.

Bibliography

55. Dos Santos, B., F. J., Cervantes, and J.B., Van Lier (2007). Review paper on current technologies for decolourisation of textile wastewaters: perspective for anaerobic biotechnology. *Bio resource Techno*, 19: 2369-2385.
56. Dwivedi, S., R. D., Tripathi, U.N., Rai, S., Srivastava, M. K., Shukla, K. K., Gupta, K. K., Tiwari, and C. P., Dwivedi, 2005. *Abst. Third International Conf. on plants and Environmental Pollution*. pp108.
57. Egbeeni, P.O.U., P.N., Okolie, O.E., Adejuyitan, A.O., Sobande, and O.K., Inyenmi (2009). Effect of industrial effluents on the growth of and anatomical structures of *Abelmoschus esculentus* (Okra). *African Journal of Biotechnology*, 8: 3251-3260.
58. Egger, K.N. (2010). Common soil bacteria key. UNBC.
59. Elango, R. G., and S., Elango (2016). Physico-Chemical Parameters of Textile Dyeing Effluent and Its Impacts with Case study. *International Journal of Research in Chemistry and Environment*, 7(1): 17-22.
60. Elango G., G., Rathika, S., Elango (2017). Physico-Chemical Parameters of Textile Dyeing Effluent and Its Impacts with Case study. *International Journal of Research in Chemistry and Environment*, 7(1): 17-24.
61. El-Komy, H.M.A. (2005). Coimmobilization of azospirillum lipoferum and bacillus megaterium for successful phosphorous and nitrogen nutrition of wheat plants. *Plant Nutr. Food Technol. Biotechnol.*, (43): 19-27.
62. Elsheikh, E.A.E. and M., Wood (1986). Soil Biology. *Biochem*, 21: 883-887.
63. EPA. (1997). Profile of the textile industry. *Environmental Protection Agency*, Washington, USA.

Bibliography

64. Erum, S. and B., Asghari (2008). Variation in phytohormone production in Rhizobium strains at different altitudes of northern areas of Pakistan. *Pak. Int. J. Agri. Biol.*, 10: 536-40.
65. Esabela, K., C., Sharma, and S. S., Chauhan (2011). Physico-Chemical profile of untreated irrigation water from Amanishah nalla, sanganer, (Jaipur). An International Quarterly Journal of Environmental Science, 5(1&2): 55-58.
66. FAO, 1985. Water Quality for Agriculture. Paper No. 29 (Rev.1) *UNESCO, Publication*, Rome.
67. Faryal, R., F., Tahir, A., Hameed (2007). Effect of wastewater irrigation on soil along with its micro and macro flora. *Pakistan J Bot.*, 39(1): 193-204.
68. Feigin, A., Ravina, I. and Shalheveth, J. (1991). Irrigation with treated sewage effluent management for environmental protection. *Advanced Series in Agricultural Sciences*, (17): 224.
69. Gadallah, M.A.A. (1999). Effects of proline and glycinebetaine on *Vicia faba* response to salt stress. *Biologia Plantarum*, 42: 249–257.
70. Garg, V.K. and P., Kaushik (2008). Influence of textile mill waste water irrigation on the growth of sorghum cultivars. *Applied Ecology and Environmental Research*, 6(2): 1-12.
71. Gauri., A.K. Singh, R.P. Bhatt, S. Pant, M.K. Bedi, and A. Naglot (2011). Characterization of Rhizobium isolated from root nodules of *Trifolium alexandrinum*. *Journal of Agriculture Technology*, 7(6):1705-1723.
72. Ghaly, A. E., R., Ananthashankar, M., Alhattab, and V.V., Ramakrishnan (2014). Production, Characterization and Treatment of Textile Effluents: A Critical Review. *Journal of chemical engineering and process technology*, 5(1): 5-11.

Bibliography

73. Gheorghe, I.F. and B., Ion (2012). The effects of air pollutants on vegetation and the role of vegetation in reducing atmospheric pollution. *Impact of air pollution on health, economy, environment and agricultural sources*, ISBN: 978-953-307-528-0.
74. Gochande, B.D. and G.S. Khansole, 2011. Morphological, cultural and Biochemical charactraizion of *Rhizabium Japonicum* sys. And *Bradyrhizabium Japenicum* of soyabean. Bioscience Discovery, 2(1): 1-4.
75. Goyal V., S., Sudesh and S., Sharma (2013). Physico-chemical analysis of textile effluents of dye and printing cluster of Bagru region, Jaipur, India. *J. Environ. Res. Develop.*, 8(1): 11-15.
76. Graham, P. H. and C. A., Parker (1964). Diagnostic features in the characterization of the root nodule bacteria of legumes. *Plant and Soil*, 20: 283-395.
77. Gupta I.C. and B. L., Jain (1992). Salinisation and alkalization of groundwaters polluted due to textile hand processing industries in Pali. *Curr. Agric.*, 16(1-2): 59-62.
78. Gupta, S.K, R.C., Gupta and A.K., Seth (1994). Reversal of clinical and dental fluorosis. Indian paediatrics, 31(4): 439.
79. Gupta and B. Ray (2005). Bioaccumulation of Cadmium, Zinc, Copper and Chromium by *Withania Somnifera*. *Nature Environ. And Poll. Tech.*, 4:131-135.
80. Hamilton, A.J., F., Stagnitti, X., Xiong, S.L., Kreidl, K.K., Benke, P., Maher (2007). Wastewater irrigation: The state of play. *Vadose Zone Journal*, 6: 823-840.
81. Hatem, I., and J., Tan (2003). Image analysis. In: Heldman, D.R. (Ed.), *Encyclopedia of Agriculture, Food, and Biological Engineering*. Marcel Dekker, New York, pp. 517–523.

Bibliography

82. Heinfling, A., M., Bergbauer and U., Szewzyk (1997). Biodegradation of azo and phthalocyanine dyes by *Trametes versicolor* and *Bjerkandera adusta*. *Appl. Microbiol. Biotechnol.*, 48: 261-266.
83. Himabindu, T. and Reddy J.K., (2005). Effect of paper and pulp mill effluents on biochemical characteristics of rice (var. Swarna mahsuri) Nature Environ. *Pollution Technol*, 4(4): 617-619.
84. Hoddy, E. 1991. Paper, pulp and Agriculture: How Industrial Waste Recycling can Benefit Farmers. *Development and Corporation*, 6: 25-26.
85. Hunger, K. (2003). Industrial Dyes: Chemistry, Properties, Applications, Wiley-VCH, Weinheim, Cambridge. Pp: 14-35.
86. Husain, J., K.C., Sharma, I., Husain, K.G., Ojha, V.K., Vaidya. (2001). Physico chemical characteristic of water from bore wells of an industrial town Bhilwara, Rajasthan: A correlation Study. *Asian Journal of Chemistry- An International Journal*, (13a): 509-512.
87. Hussain, J, I., Husain (2002). Study on the Impact of Textile Wastewater on The Groundwater Quality of Villages Close To River Banas, Rajasthan, India, India. *Journal of Environmental Protection*. (23b): 1038-1044.
88. Hussain, M., M., Ashraf., M., Saleem and F. Y., Hafeez (2002). Isolation and Characterization of Rhizobial Strains from Alfalfa. *Pak. J. Agri. Sci.*, 39: 32-34.
89. Heritage, J., E.G.V., Evans, R.A., Killington (1999). The microbiology of soil and nutrient cycling. In: Microbiology in action. Cambridge University Press, U.K. Pp 289.
90. Imtiazuddin, S.M., M., Majid, Khalil and A., Mallick (2012). Pollutants of wastewater characteristics in textile industries. *Journal of Basic & Applied Sciences*, 8: 554-556.

Bibliography

91. Indiragandhi, K., M., Kannahi and M., Gomathi (2014). Screening and physico - chemical characterization of textile effluent and their effect on *Vigna mungo* growth. *International Journal of current Microbiology and Applied science*, 3(5): 51-58.
92. International Seed Testing Association (1985). *Seed Sci. & Tech.*, 13: 299-355.
93. Iram, A. and T.I., Khan (2018). Analysis of Soil Quality Using Physico-Chemical Parameters with Special Emphasis on Fluoride from Selected Sites of Sawai Madhopur Tehsil, Rajasthan. *International Journal of Environmental Sciences & Natural Resources*, 12(5): 125-132.
94. Islam, M. M., K. Mahmud, O. Faruk, and M. S. Billah (2011). Textile Dyeing Industries in Bangladesh for Sustainable Development. *International Journal of Environmental Science and Development*, 2 (6): 428-436.
95. Jain, R.K. and S., Kumari (1990). Effect of saree printing industry effluent on seed germination, seedling growth and total biomass of *Spinacea oleracea*. *Acta Ecologica*, 12(1): 19-22.
96. Jaishree and T.I. Khan (2013). Physicochemical analysis of textile waste water around agricultural fields in Sanganer town, Jaipur. *Global Journal of Bioscience and Biotechnology*, 2 (3): 458-460.
97. Javed, K. and B., Asghari (2008). Potential allelopathic effects of sunflowers on microorganisms. *Afri. J. biotech*, 7(22): 4208-4211.
98. Jolly, Y.N., A. Islam, and A.I., Mustafab (2012). Impact of dyeing industry effluent on soil and crop. *Universal Journal of Environmental Research and Technology*, 2(6): 560-568.
99. Joshi N, and A., Kumar (2011) Physico-chemicalanalysis of soil and industrial effluent of Sanganer region of Jaipur, Rajasthan. *Research Journal of Agricultural Science* 2: 354-356.

Bibliography

100. Joshi, V.J. and D.D., Santani (2012). Physicochemical characterization and heavy metal concentration in effluent of textile industry. *Universal Journal of Environmental Research and Technology*, 2(2):93-96.
101. Junior, M.A., A.S.T., lima, J.R.F., Arruda and D.L., Smith (2005). Effect of root temperature on nodule development of bean, lentil and pea. *Soil Biol. Biochem.*, (37): 235-239.
102. Kadioglu, A. and O.F., Algur (1990). The effect of vinasse on the growth of Helianthus annuus and Pisum sativum: part I-The effects on some enzymes and chlorophyll and protein content. *Environ. Poll.*, 67: 223-232.
103. Kanan, V. R., Ramesh and C., Sasikumar (2005). Study on ground water characteristics and the effects of discharged effluents from textile units at Karur District. *Journal of Environmental Biology*, (26): 269-272.
104. Kanan, A. H., S.S., Marine, F., Raihan, M., Redwan, and M. D., Miah (2014). Textile effluents change Physiochemical parameters of Water and Soil threat for Agriculture. *African Journal of Agronomy*, 10: 219-223.
105. Kannaiyan, S. (2001). Use of Poor Quality Water and Sugar Industrial Effluent in Agriculture. Proceeding of National seminar on use of a Poor Quality water and sugar industrial effluent in agriculture. Held on February 2001 pp. 1-23.
106. Karim Md. E., K., Dhar, Md. T., Hossain (2015). Physico-Chemical and Microbiological Analysis of Textile Dyeing Effluents. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 9(7): 41-45.
107. Kathirvel, P. (2012). The Effect of dye factory effluent on growth, yield and biochemical attributes of Bengal gram (*Cicer arietinum* L.). *International Journal of Applied Biology and Pharmaceutical Technology*, 3(1): 146 -150.

Bibliography

108. Kaur, A., S., Vats, S., Rekhi, A., Bhardwaj, J., Goel, R., Tanwar, and K., Gaur (2010). Physico-chemical analysis of the industrial effluents and their impact on soil microflora. *International Society for Environmental Information Sciences*, (2): 595-599.
109. Khai, N.M., P.T., Tuan, C.N., Vinh, I., Oborn (2008). Effects of using wastewater as nutrient sources on soil chemical properties in periurban agricultural systems. *VNU Journal of Science. Earth Sci.*, 24: 87-95.
110. Khan, T.I., N., Singh, R., Yadav, and D.M., Solomon, (2001). Heavy metals in the vegetables from textile industrial area Sanganer, Stress and Environmental Plant Physiology, *Avishkar Publication*, Jaipur: 51-55.
111. Khan, N.A., L. Gupta, S., Javid, S., Singh, M., Khan, A., Inam and Samiullah (2003). Effects of sewage waste water on morphophysiology and yield of *Spinacia* and *Trigonella*. *Ind. J. Plant Physiol.*, 8: 74-78.
112. Khan, M. Gufran, G., Daniel, M., Konjit, A., Thomas (2011). Impact of Textile Waste Water on Seed Germination and Some Physiological Parameters in Pea (*Pisum sativum* L.), Lentil (*Lens esculentum* L.) and Gram (*Cicer arietinum* L.). *Asian journal of plant sciences*, 10(4): 269-273.
113. Khan, S. and N., Mathur (2013). Microbial Flora of Textile Effluents Areas of Western Rajasthan. *International journal of plant, animal and environmental science*, (3): 127-129.
114. Khan, S., and N., Mathur (2015). Impact of Textile Effluents on Water bodies of Western Rajasthan. *International Journal of Advanced Research in Biological Sciences*, 2(3): 183-188.
115. Kibria, M.G., M., Islam, M., Alamgir (2012). Influence of wastewater irrigation on heavy metal accumulation in soil and plant. *International Journal of Applied and Natural Sciences*, 1(1): 43-54.

Bibliography

116. Kolhe, A.S. and V.P., Pawar (2011). Physico-Chemical Analysis of Effluents from dairy industry. *Recent Research in Science and Technology*, 3(5): 29-32.
117. Krichner and Buchanan (1926). *Rhizobium joponicum* syn. *Brodyrhizobium joponicum*. *Int. J. Syst. Bacteriol.* 30: 335 742.
118. Krishna, K., and S., Leelavathi (2002). Toxicity of sugar factory effluent to germination, vigour index and chlorophyll content of paddy. *Nature, Environment and Pollution Technology*, 1(3): 249-253.
119. Kulandaivel, S., P., Srivaishnavi, P., Kaleeswari, and P., Mohanapriya (2014). Degradation and adsorption of industrial effluents by consortium of microbes isolated from agro forestry soil. *International Journal of Current Microbiology and Applied Sciences*, 3(12): 883-894.
120. Kumawat, D.M., K., Tuli, P., Singh, and V., Gupta (2001). Effect of dye industry effluent on germination and growth performance of two rabi crops. *Journal of Ecobiology*, 13(2): 89-95.
121. Kumar, G., S., Banu and Kannan (2006). Effects of textile mill effluents on seed germination, seedling growth and pigment content on *Arachis hypogaea*. *Journal of Ecobiology*, 19(1): 19-22.
122. Kumari B. S., M. R., Ram and K. V., Mallaiah (2010). Studies on nodulation, biochemical analysis and protein profiles of Rhizobium isolated from Indigofera species. *Malaysian Journal of Microbiology*, 6(2): 133-139.
123. Kuske C. R., L.O., Ticknor, M.E., Miller, J.M., Dunbar (2002). Comparison of soil bacterial communities in Rhizospheres of three plant species and the interspaces in an arid grassland. *Applied and Environmental Microbiology*, 68(4): 1854-1863.

Bibliography

124. Lalitha, S. and Immanuel S P., (2013). Biochemical characterization of Rhizobium and its impact on black gram and green gram plants. *International Journal of Current Science*, 9:1-6.
125. Lenin M., Mariyappan K.S. and Thamarikannan M.R (2014). Effect of sago factory effluent on seed germination and seedling growth of gingelly (*sesamum indicum L.*) varieties. *Int. J. Life Sc. Bt. & Pharm. Res.*, 3(1): 2250-3137.
126. Lindemann, W.C. (2008). Nitrogen fixation by legumes, cooperative Extension Service College of Agriculture and Home Economics, New Mexico State University, *Guide A-129*.
127. Lindsay, W.L. and W.A., Norvell (1978). Development of DTPA soil test for zinc, Manganese, and copper. *Soil, Sci. SOC AMJ*, 42: 421-428.
128. Lowry, O.H., N.J. Rosbrough, A.L. Farr, and R.J. Randall (1951). *Journal of Biological Chemistry*, 193: 265-275.
129. Mahale, G., K., Bhavani, R. K., Sunanda and M., Sakshi (1999). Standardization dyeing conditions for African Marigold. *Man-made textiles in India*, 42(11): 453-458.
130. Mahajan S. and D. Billore (2014). Seasonal Variations and Assessment of Water Quality of Nagchoon pond of Khandwa District (M.P.) India. *Current World Environment*, 9(3): 829-836.
131. Maheshwari N. (2012). Influence of different pollutants on soil bacteria of kota district ph.D thesis, university of Kota.
132. Mahna S K, R., Garg and M., Parvateesam (2000). Cultural and Biochemical Characteristics of root nodule bacteria from induced mutants of *Vigna mungo L.* *Seed Pathology, Printwell publ, Jaipur*. pp: 417-421.

Bibliography

133. Malaviya P., R., Hali, N., Sharma (2012). Impact of dyeing industry effluent on germination and growth of pea (*Pisum sativum*). *J. Environ. Biol.*, 33: 1075-1078.
134. Malik A. (2017). Physico-chemical and microbial analysis of the soil contaminated by textile industries located in sanganer industrial area, Jaipur. *International Journal of Scientific & Engineering Research*, 8(10): 1687-1693.
135. Manikandan, P., N., Palanisamy, R., Baskar, P., Sivakumar, and P., Sakthisharmila (2015). Physicochemical analysis of textile industrial effluents from Tirupur city, in India. *International Journal of Advance Research in Science and Engineering*, 4(2), 93-104.
136. Manivasakam, N (1987). Informations to be obtained from industries, In: Industrial Effluents – Origin, Characteristics, Effects, Analysis and Treatment. *Sakthi Publications*, Coimbatore: 348-387.
137. Mathur, N. and A., Kumar (2013). Physico-chemical characterization of industrial effluents contaminated soil of sanganer. *Journal of Emerging Trends in Engineering and Applied Sciences*, 4 (2): 226-228.
138. Medhi, U.J., A.K., Talukdar, and S., Deka (2011). Impact of paper mill effluent on growth and development of certain agricultural crops. *Journal of Environmental Biology*, 32: 185-188.
139. Mehta R. and K., Yadav (2013). Soil contamination due to Textile Effluent- Case study on the Printing cluster of Jaipur. *Journal of the Textile Association*; 367-370.
140. Mehta A. and N. Bhardwaj (2012). Phytotoxic effect of industrial effluents on seed germination and seedling growth of *Vigna radiate* and *Cicer arietinum*. *Global Journal of Bio-science and Biotechnology*, 1: 1-5.

Bibliography

141. Menna, P., M., Hungria, F.G., Barcellos, E.V., Bangel, P.N., Hess, and E., Martinez-Romero (2006). Molecular phylogeny based on the 16S rRNA gene of elite rhizobial strains used in Brazilian commercial inoculants. *Systematic and Applied Microbiology*, 29: 315-332.
142. Metcalf, Eddie (2003). Wastewater Engineering Treatment and Reuse, From Edition. New York, USA: McGraw Hill.
143. Michael, J. S., and P. H., Graham (2006). Root and stem nodule bacteria of legumes. *Prokaryotes*, 2: 818- 841.
144. Mishra, P. and A.K. Bera, (1995). Effect of tannery effluent on seed germination and early seedling growth in wheat. *Seed Res.*, 23: 129-131.
145. Mishra, P., and R., Soni (2016). Analysis of dyeing and printing waste water of Balotara textile industries. *Int. J. Chem. Sci.*, 14(4): 1929-1938.
146. Mohan, S.V., N.C., Rao, K.K., Prasad, J., Karthikeyan. (2002). Treatment of simulated Reactive Yellow 22 (Azo) dye efluents using Spirogyra species. *Waste Manag*, 22:575–582.
147. Mojiri A., (2011). Effects of Municipal waste water on physical and chemical properties of saline soil, *J. Boil. Environ. Sci.*, 5(14): 71-76.
148. Moore, S. and Stein, W.H. 1948. Photometric methods for use in the chromatography of amino acids. *Journal of Biological sciences*, 176: 367-388.
149. Muamar AL., M., Tijane, EL., Shawqi , El., Abdellah Housni , A., Zouahri, B., Mohammed (2014). Assessment of the impact of wastewater use on soil properties. *J. Mater. Environ. Sci.*, 5 (3): 747-752.

Bibliography

150. MurKumar, C.V. and Chavan, P.D. 1987. Influence of Water Pollution on Germination of Gram (*Cicer arietinum* L.). In: Current pollution research in India, 233-238.
151. Naaz S., and S.N., Pandey (2010). Effects of industrial waste water on heavy metal accumulation, growth and biochemical responses of lettuce (*Lactuca sativa* L.). *Journal of Environmental Biology*, 31: 273-276.
152. Nagajyoti, P.C., K.D. Lee, TVM., Sreekanth (2010) Heavy metals, occurrence and toxicity for plants: a review. *Environ Chem Lett.*, 8: 199-216.
153. Ndour N, E., A.B., Guissé, M., Seck, M., Khouma, A., Brauman (2008). Impact of irrigation water quality on soil nitrifying and total bacterial communities. *Biology and Fertility of Soils*, 44: 797-803.
154. N., Nelson (1944). *Anal. Chem.*, 3-426.
155. Neelam, S. and Sahai, R. 1988. Effect of fertilizer factory effluent on seed germination, seedling growth, pigment content and biomass of *Sesamum indicum* L. *J. Environ. Biol.*, 9: 45-50.
156. Nicholson, F. A., S. R., Smith, B. J., Alloway, C., Carlton-Smith and B. J., Chambers (2003). An inventory of heavy metal input to agricultural soils in England and Wales. *Science of the Total Environment*, 311: 205–219.
157. Nirgude, N. T., S. Shukla and A., Venkatachalam (2013). Physico-chemical analysis of some industrial effluents from vapi industrial area, Gujarat, India. *Rasayan J. Chem.*, 6(1): 68-72.
158. Niroula, B. (2003). Comparative Effects of Industrial Effluents and sub-metropolitan Sewage of Biratnagar on Germination and seedling growth of Rice and Blackgram. *Our Nature*, 1: 10-14.

Bibliography

159. Oblisami, G., (1974). Studies on Rhizobium and nodulation pattern in a forage legume, *Clitoria ternate* isolated from paddy field. *Indian National Science Academy Proceeding B*, 40: 618-623.
160. Oblisami, G. (1995). In vitro growth of five species of ectomycorrhizal fungi. *Euro J for Path* 1-7: 204– 210.
161. Olson and Hodges (1987). Recommended dietary intakes (RDI) of vit. c inhumans *Ann J. Clin. Nutri* 45,pp.693.
162. Panasker, D.B. and R.S., Pawar (2011),“Effect of textile mill effluent on growth APHA: *Standard methods for the examination of water and wastewater 19th Edn.* Washing DC USA (1995).
163. Parameswari M. (2014). Effect of Textile and Dye Effluent Irrigation on Germination and its Growth Parameters of Green Gram, Black Gram and Red Gram. *International Journal of Environmental Science and Toxicology Research*, 2(1): 6-10.
164. Patel H., and S., Pandey (2008). Physico-chemical characterization of textile chemical sludge generated from various CETPS in India. *J. Environ. Res. Develop.*, 2(3): 329-339.
165. Patel, R., K., Tajddin, A., Patel, B., Patel (2015). Physico-Chemical Analysis of Textile Effluent. *International Journal of Research and Scientific Innovation*, 2(5): 33-37.
166. Patil, S.N., D., Yeole and N.D., Wagh (2014). Evaluation of surface water quality and its suitability for drinking and agricultural use in Jalgaon district, Maharashtra, India. *Nature Env. Poll. Tech.*, 13(1): 133-139.
167. Paul, S. A., S. K., Chavan and S. D., Khambe (2012). Studies on characterization of textile industrial waste water in Solapur city. *Int. J. Chem. Sci.*, 10(2): 635-642.

Bibliography

168. Pawar V. A., P. R. Pawar, A. M. Bhosale and S. V. Chavan (2014). Effect of *Rhizobium* on Seed Germination and Growth of Plants. *Journal of Academia and Industrial Research*, 3(2): 84-88.
169. Pescod M.B. (1992). Wastewater treatment and use in agriculture. *FAO Irrigation and Drainage Paper*, No. 47: 113.
170. Prabha, S., A., Goyal, P., Mazumder, A. L., Ramanathan and M., Kumar (2016). Assessment of the impact of textile effluents on microbial diversity in Tirupur district, Tamil Nadu. *Applied Water Science*, 7(5): 2267-2277.
171. Pragasam, A. and B. Kannabiran, 2001. Growth and physiological activity of green gram under effluent stress, Adv. Plant Sci., pp: 14-547.
172. Pribac, C. and A. Ardelean (2008). In vitro culture of *Trigonella foenumgraecum* plantules and their anatomic characterization. Proceedings of the EMC 14th European Microscopy Congress, sept.1-5, Springer, Berlin, Heidelberg, 181-182.
173. Purohit, S.S. and A., Kumar (1998). Plant Physiology, Agro Botanical Publishers (Incuial/Agro Botonica Bikone): 289.
174. Rai, S., A.K., Chopra, C., Pathak, D. K., Sharma, R., Sharma, and P. M., Gupta (2011). Comparative study of some physicochemical parameters of soil irrigated with sewage water and canal water of Dehradun city, India. *Archives of Applied Science Research*, 3 (2): 318-325.
175. Rajeswari, M., K., Kalaichelvi, S., Manian and J., Indramuthu (2005). Irrigational impact of dye house effluent on plant growth and soil characteristics. *J. IndI. PolIn. Ctrl.*, 21(2): 299 - 304.
176. Rana, S and K. Kumar (2017). Study of Phytotoxic effect of textile wastewater on seed germination and seedling growth of *Triticum aestivum*. *International Journal of BioSciences and Technology*, 10(8): 58-66.

Bibliography

177. Ramasubramanian, V., V., Ravichandran, and N., Kannan (1993). Analysis of industrial effluents and their impact on the growth and metabolism of *Phaseolus mungo* L. *Comm. Soil Sci. Plant Analysi*, 24: 2241-2249.
178. Ramachandra, T.V, S.M.D., Chandran, N.V., Joshi, R., Rajinikanth and R., Kumar (2012). Water, soil and sediment characterization: Sharavathi river basin, Western Ghats., ENVIS Technical Report: 21, Energy & Wetlands Research Group, Centre for Ecological Sciences, Indian Institute of Science, Bangalore 560 012.
179. Ramya, S., R. K., Pradeep, S., Murugesan, and S., Anitha (2017). Effect of Textile Effluent on Seedling Germination, Growth and Biochemical Characteristics of *Arachis hypogaea* l. Variety K6. *International Journal of Pharma Research and Health Sciences*, 5(4): 1805-09.
180. Rao A. V., B. L., Jain and I. C., Gupta (1993). Impact of textile Industrial effluents on agricultural land – A case study, Indian J. *Environ Health*, 35 (2): 132 – 138.
181. Rasool S, B., Sharma and S., Rasool (2015). Isolation and characterization of Rhizobium sp. from a wild legume from BGSBU campus of District Rajouri, J&K. *International Journal of Agricultural Sciences*, 5: 407-13.
182. Rathod, M. C., B., Senjaliya and D.A., Dhale (2015). Effect of textile dye on seed germination of chickpea. *International Journal of Recent Scientific Research*, 6(3): 2938-2943.
183. Ravi, D., R.Parthasarathy, V., Vijaybharti and S., Suresh (2014). Effect of textile dye effluent on soyabean crop journal of pharmaceutical. *Chemical and Biological Sciences*, 2(2): 111-117.
184. Richards GA, Theron AJ, Van Rensburg CE, Van Rensburg AJ, Van der Merwe CA, Kuyl JM, Anderson R. (1990). Investigation of the effects of oral administration of vitamin E and beta-carotene on the chemiluminescence responses and the frequency of sister chromatid exchanges in circulating leukocytes from cigarette smokers. Am Rev Respir Dis 142:648–654.

Bibliography

185. Robinson, T., B., Chandranand and P., Nigam (2002). Textile effluent decolorization and dye-adsorbed agricultural residue biodegradation. *Biores. Tech.*, 84:299-301.
186. Sacks, M., and N., Bernstein (2011). Utilization of reclaimed wastewater for irrigation of field-grown melons by surface and subsurface drip irrigation. *Isreal Journal of Plant Science*, 59: 159–169.
187. Sadasivam, S., A., Manikam (1992) Biochemical Methods for Agricultural Sciences. *Wiley Eastern Limited*, New Delhi, India.
188. Sadowsky, M.J., H.H., Keyser and B.B., Bohlool (1983). Biochemical characterization of fast-and slow-growing rhizobia that nodulate soybeans. *Int. J. Syst. Bacteriol*, 33(4): 716-722.
189. Sah, S.K., J.P., Sah and V.A., Lance (2000). Industrial Effluents and their Use in Agriculture along the Narayani River, Nawalparasi, Nepal. In: Environment and Agriculture: At the Crossroad of the New Millennium (eds.) Jha, P.K., S.B. Karmacharya, S.R. Baral and P. Lacoul. Ecological Society (ECOS), Nepal, pp. 456-466.
190. Sahai, R., S., Jabeen and Saxena, K. (1983). Effect of distillery waste on seed germination seedlings growth and pigment content of rice. *Indian Journal of Ecology* 10(11): 7-10.
191. Sammy, G.K., N.C. Singh, K. Jitman and V. Sweeney. 1995. Industrial Wastewater Management in the Caribbean Region. *Industry and Environment* 18, (2-3): 88-92.
192. Saravanamoorthy, M. D. and B. D. Ranjitha Kumari (2007). Effect of textile water on morphology and yield on two varieties of peanut (*Arachis hypogea* L.). *Journal of Agricultural Science and Technology*, (3): 335-343.

Bibliography

193. Saraswat S, JPN, Rai (2011). Prospective application of *Leucaena leucocephala* for phytoextraction of Cd and Zn and nitrogen fixation in metal polluted soils. *Int. J. Phytorem*, 13: 271-288.
194. Sarnaik, S. and P., Kanekar (1995). Bioremediation of color of methyl violet and phenol from a dye industry waste effluent using *Pseudomonas* sp. isolated from factory soil. *Journal of Applied Bacteriology*, 79: 459-469.
195. Sasikala, T and Poongodi. N. (2013). Impact of Dye Effluent on Seed Germination of Black gram (Vigna Mungo.L.hepper). Indian Journal of Applied Research. 3(8).
196. Sathiyaraj, G., K. C., Ravindran, Z.H., Malik (2017). Physicochemical characteristics of textile effluent collected from Erode, Pallipalayam, and Bhavani polluted regions, Tamil Nadu, India. *Journal of Ecobiotechnology*, 9: 1-4.
197. Sawyer, C.C. and P.L., Mc Carty (1978). Chemistry for Environmental Engineers, New York: McGraw Hill: 331-514.
198. Sen, D.N. (1977). Environment and Seed germination of Indian Plants. Chronica Botanica Co., New Delhi.
199. Sharma, K.C., I., Husain, J., Husain, K.G., Ojha, (2001). Ground water quality of an industrial town Bhilwara, Rajasthan. *Journal of Environmental and Pollution*, 8(1): 109-114.
200. Sharma, R. K., M. Agrawal. and F. M., Marshall (2007). Heavy metals contamination of soil and vegetables in suburban areas of Varanasi, India. *Ecotoxicology and Environmental Safety*, 66: 258-266.
201. Sharma, S.K., N. Sharma and Y. Khandelwal (2014). Comparative analysis of physio chemical characteristics of dyeing industry effluent between Sanganer and Bagru printing clusters, Jaipur (Rajasthan). *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 8(4): 15-18.

Bibliography

202. Shahzad, F., M., Shafee, F., Abbas, S., Babar, M., Tariq and Z., Ahmad (2012) Isolation and Biochemical Characterization of Rhizobium meliloti from Root Nodules of Alfalfa (*Medico sativa*). *The Journal of Animal and Plant Sciences*, 22: 522-524.
203. Shanmughavel, P. (1993). Impact of sewage, paper and dye industry effluents on germination of greengram and maize seeds. *J. Ecobiol.*, 5(1): 69-71.
204. Sheikh, K.H. and M., Irshaad (1980). Wastewater Effluents from a Tannery: Their Effects on Soil and Vegetation in Pakistan. *Environ Conserv.*, 79(4): 319-324.
205. Shi, W., J., Becker, M., Bischoff, R. F., Turco, and A. E., Konopka (2002). Association of microbial community composition and activity with lead, chromium, and hydrocarbon contamination. *Applied and Environmental Microbiology*, 68(8): 3859–3866.
206. Shivakumar, D., S., Srikanthswamy, B.M., Kiran, and S., Sreenivasa (2012). Study of impacts of industries on soil characteristics of Mysore city, India. International Journal of Geology. *Earth and Environmental Sciences*, 2(2): 25-33.
207. Singh, D. K., D. Kumar, V. P. Singh and J. Environ, (1985). Growth and physiological activity of green gram under effluent stress. *Biol.*, pp: 6 – 31.
208. Singh, B., K., Ravneet and S., Kashmir (2008). Characterization of Rhizobium strain isolated from the roots of *Trigonella foenumgraecum* (fenugreek). *Afri. J. Biotech*, 7 (20): 3671-3676.
209. Sisodia, G.S. and Bedi, S.J. 1985. Impact of chemical industry effluent on seed germination and early growth performance of wheat. *Indian J. Ecol.* 12: 187-192.

Bibliography

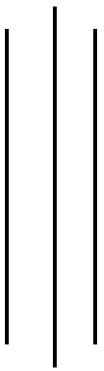
210. Smrithi, A., A., Bhaigyabati and K., Usha (2012). Bioremediation potential of *Brassica juncea* against textile disposal. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 3(2): 395.
211. Soffe R.E., In. The Agricultural Notebook, 19th Edition. Black well Science, Oxford, (1995).
212. Somashekhar, R.K., M.T.D., Gowda, Shettigar, and K.P. Srinath (1984). Effect of industrial effluent on crop plants. *Indian J. Environ. Hlth.*, 26: 136-146.
213. Sprent, J. I. (2001). *Nodulation in Legumes*, Kew, UK: Royal Botanic Gardens.
214. Sriram, N. and D. Reetha (2015). Isolation and characterization of dye degrading bacteria from textile dye effluents. *Central European Journal of Experimental Biology*, 4 (2), 5-10.
215. Srivastava, N., and Sahai, R. 1987. Effect of distillery wastes on the performance of *Cicer arietinum* L. *Environ. Pollut.* 43: 91-102.
216. Stefanowicz A.M., M., Niklinska, R., Laskowski (2008) Metals affect soil bacterial and fungal functional diversity differently. *Environ Toxicol Chem*, 27(3): 591-598.
217. Suriyanarayanan S., G., Jessen, L., Divya and S., Balasubramanian (2012). Effect of waste paper industry effluents on growth of tree seedlings. *J. Environ. Res. Develop.*, 7(2A): 1117-1126.
218. Swaminathan, K. and P., Vaidheeswaran (1991). Effect of dyeing factory effluents on seed germination and seedling development of groundnut (*Arachis hypogaea*). *J. Environ. Biol.*, 12: 353-358.
219. Tahir, M. and Mughal K. (2012). Pakistan Textile industry and the neighboring countries (A Globalization effect). *Far East Journal of Psychology and Business*, 8 (2): 66-70.

Bibliography

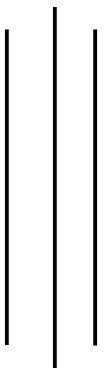
220. Thakur, A.K. and K.J., Singh (2014). Heavy metal Cd affecting nodulation and leghaemoglobin proteins in soybean and chickpea. *BioMed Research the Open Access Publisher*, 1(1): 1-6.
221. Thoker F. A., S., Manderia and K., Manderia (2012). Impact of Dye Industrial Effluent on Physicochemical Characteristics of Kshipra River, Ujjain City, India. *International Research Journal of Environment Sciences*, 1(2): 41-45.
222. Trivedy, R. K. and Goel, P. K. (1986). Chemical and Biological method for water pollution studies. *Environmental publication* (Karad, India), 6: 10-12.
223. Uaboi-Egbenni P.O., P.N., Okolie, O.E., Adejuyitan, A.O., Sobande, O., Akinyemi (2009). Effect of industrial effluents on the growth and anatomical structures of *Abelmoschus esculentus* (okra). *Afr. J. Biotechnol*, 8: 3251-3260.
224. Varma, L. and J. Sharma (2010). Analysis of Physical and Chemical Parameters of Textile Waste Water. *Journal of International Academy of Physical Sciences*, 15 (2): 269-276.
225. Varma, L. and J., Sharma (2012). Effect of dairy and textile waste water on growth of plant wheat. *Rasayan Journal*, 5(3): 351-355.
226. Vijayarengan, P. and A.S., Lakshmanachary (1993). Effect of textile mill effluent on growth and development of green gram seedlings. *Adv. Plant Sci.*, 6: 359-365.
227. Vijayakumari, B. (2005). Response of *Eleusine coracana* to textile dyeing industry effluent. *Journal of Ecobiology*, 17: 79-82.
228. Vijayaragavan M., J., Surshkumar, A, Natarajan, P, Vijayarengan, S., Sharavanan, C., Prabhahar (2011). Soil irrigation effect of sugar mill effluent on changes of growth and biochemical content of *Raphanus sativus* L. *Curr Bot*, 2:09-13.

Bibliography

229. Vincent, J.M., (1970). A manual for the practical study of the root nodule bacteria, I.B.P. *hand Book Blackwell scientific publications Oxford.*
230. Walkley, A., I.A., Black (1934). An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science*, 37: 29 - 38.
231. WHO (2006). Guidelines for the safe use of wastewater, excreta and greywater, 2: Wastewater Use in Agriculture, World Health Organization, Geneva.
232. WHO (2006). Guidelines for technologies for water supply systems in small communities (World Health Organization, CEHA).
233. Yang, C., M.C., Jared, and J. Garrahan, (2005). Electrochemical coagulation for textile effluent decolorization. *J. Hazard. Mater B*, 127: 40-47.
234. Yousaf, A., S.M., Ali, and A.Z., Yasmin (2010). Germination and early growth response of Glycine max varieties in textile and paper industry effluents. *Pakistan Journal of Botany*, 42(6), 3857-3863.



PUBLICATIONS



Impact of Dye Effluent on Seed Germination, Seedling Growth and Chlorophyll Content of Soybean (*Glycine max L.*)

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Abstract: Textile dyeing industry is one of the major water consuming and high polluting industries in India. Untreated industrial effluent discharged into ecosystem pose a serious problem to the aqua living organisms, plants and human being. Effluent in higher concentration affects the soil and causes heavy damage to crop growth. In the present study effects of dyeing effluent on seed germination and seedling growth of soybean (*Glycine max L.*) has been carried out at different concentration (20, 40, 60, 80, 100%) of the effluent collected from dyeing industries at Kaithun region, Kota and control was maintained. Seed germination percentage of *Glycine max* was not significantly affected at 20% treatment level where as higher concentration of dyeing effluent negatively affects the germination percentage of seeds. Seeding growth (Shoot and Root lengths) and Chlorophyll content (Chlorophyll a and b) have found to increase at 20% and 40% of treatment level. The root length was found to increase 4.4% and 2.2% and shoot length was found to increase 1.5% and no change at treatment level 20% and 40% respectively than control. Due to increase in concentration of effluent at 60% treatment level and above a gradual decline of seedling growth was observed. At 100% treatment level root length and shoot length were decrease 57.7% and 58.7% respectively in comparison to control. Chlorophyll content was also adversely affected due to higher concentration of dyeing effluent. The present study revealed that *Glycine max L.* (soybean) is susceptible to dyeing industrial effluent.

Keywords: Dye Industrial Effluent, Physico-chemical analysis, Soybean seeds, Germination percentage, Seedling growth, Chlorophyll a and b

1. Introduction

The unplanned rapid industrial growth is the main cause for the environmental pollution. Each industry is associated with an emission of many pollutants. Textile industries are large industrial consumers of water as well as producers of waste water with the increased demand for textile products. The most potential and hazardous source of water and soil pollution are industrial effluents. One major group of contaminants in effluent is dyes from dying and printing industries which make it aesthetically unacceptable and toxic depending on the chemicals. The waste water from textile dying industries is a complex mixture of many polluting substances ranging from organo-chloride based waste to heavy metals associated with dye and dying process (Correia et al. 1994). These contain heavy metals poisonous compounds and nutrients which affect plant and soil in number of ways. These toxic chemicals presents in effluent caused reduction in cell activities, retardation of growth, various deficiencies and diseases when accumulated in cells of living being (Patel et al. 2008 and Naik et al. 2009). Industrial effluents are constantly adding up toxic substances into the ground water reservoir at a very high rate especially in industrial zones (Babiker et al. 2004). The industrial pollutants caused the alteration in Physico-chemical and biological properties of the environment. Dye waste water has also been found toxic to several crop plants (Parameswari M. 2014). The presence of heavy metals and toxic chemicals will show detrimental effects on the development of plants, germination process and growth of seedlings (Singh et al. 2004 and Nath et al. 2007). Effluent in higher concentration affect the soil and causes heavy damage to the crop growth conditions. The use of such

waste water in irrigation system definitely provides some nutrients to enhance the fertility of soil but it also deposits toxicants that change soil properties in the long run. The low amount of O₂ in dissolved form due to the presence of high concentration of solid in the effluent reduces the energy supply through anaerobic respiration resulting in restriction of growth of seedlings (Sazena et al. 1986). The improper and indiscriminate disposal of textile effluents in natural waters and land in posing serious problems (Kaushik et al. 2004). The exposure of lower concentration of effluent to the seedling shows growth promotion, over all development of the seedling and chlorophyll content. Reduction in seed germination percentage at higher concentration of effluent may be due to the higher amounts of solids presents in the effluent, which causes changes in osmotic relationship of the seed and water (Prabhakar et al. 2006). In the present study an attempt was made to analyze the impact of dyeing effluent, collected from dying and printing industries at Kaithoon region, Kota, on some growth parameters of soybean (*Glycine max L.*)

2. Material and Methods

Experimental Plant- Soybean (*Glycine max L.*)

Soybean is a leguminous vegetable of the pea family that grows in tropical, subtropical, and temperate climates. It consists of more than 36% protein, 30% carbohydrates, and excellent amounts of dietary fiber, vitamins, and minerals. It also consists of 20% oil, which makes it the most important crop for producing edible oil. Soybean produces significantly more protein in per acre than most other uses of land. Soy varies in growth and habit. The height of the plant varies from less than 0.2 to 2.0 m (0.66 to 6.56 ft). Soybean

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contains symbiotic bacteria called *Rhizobia* within nodules of their root system. These bacteria have the special ability of fixing nitrogen from atmosphere.

1) Collection of Effluent sample-

The effluent samples were collected from small dying and printing units from kaithoon region, Kota, Rajasthan. Samples were collected in sterilized wide mouth plastic bottles and were stored at 4° C temperature to avoid any changes on its characteristics. To evaluate the effects of dyeing effluent on *Glycine max L.*, solution of different concentrations of effluent were prepared.

Treatment Level

C- Control (100% Distilled water)
 T₁- Effluent: Distilled water (20% + 80%)
 T₂- Effluent: Distilled water (40% + 60%)
 T₃- Effluent: Distilled water (60% + 40%)
 T₄- Effluent: Distilled water (80% + 20%)
 T₅- Effluent (100%)

For germination tests, seeds were sterilized with 0.1% w/v mercuric chloride ($HgCl_2$) solution for 5 minutes to remove microbes and then washed three times with sterile distilled water. 20 seeds of *Glycine max L.* were placed in sterilized glass Petri dishes of uniform size lined with filter paper discs. These filter discs were then moistened with 5 ml of distilled water for control and with the same quantity of various concentrations of the textile effluent (20%, 40%, 60%, 80%, 100%). The Petri dishes were incubated at room temperature. 5 ml of respective dilutions were sprayed for consecutive 6 days. Germination was recorded daily. All the levels were carried out in triplicate. On the 7th day, germination percentage was calculated and various growth parameters and chlorophyll content were evaluated on 14th day of the experiment.

- **Germination percentage-** The formula given by Rehman et al.(1998) was used to estimate germination percentage.

$$\text{Germination percentage} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds}} \times 100$$

- **Root and Shoot length-** Length of root and shoot of seedlings were calculated by using the standard centimeter scale.

- **Vigour index-** The formula suggested by Abdul-Baki and Anderson (1973) was used to calculate vigour index.
 $\text{Vigour index} = \text{germination percentage} \times (\text{root length}^* + \text{shoot length}^*)$

(* indicate that length of root and shoot in cm.)

- **Fresh and Dry Weight of seeds** – Ten seeds of each treatment were weighted in order to determine the fresh weight and then dried in oven at 80° C for 24 hrs. to obtain dry weight. Fresh weight and Dry weight were recorded in mg.

- **Chlorophyll estimation-** The estimation of chlorophyll content was done according to Arnon's method (1949).

$$\text{Chlorophyll a (mg/g)} = \frac{12.7 A_{663} - 2.69 A_{645}}{A \times 1000 \times W} \times V$$

$$\text{Chlorophyll B (mg/g)} = \frac{22.9 A_{645} - 4.68 A_{663}}{A \times 1000 \times W} \times V$$

Where

A_{663} = Absorbance at wavelength 663 nm

A_{645} = Absorbance at wavelength 645 nm

V= Volume of the extract in ml

A = Length of light path in the cuvette (1cm)

W= Fresh weight of the samples in gm.

3. Results and Discussion

Table 1 is showing effect of different dilution percentage of dying and printing effluent on germination percentage, vigour index, root length, shoot length, fresh and dry weight, chlorophyll a and b content in *Glycine max L.*

Germination percentage in untreated seedling (control) of *Glycine max* was 93% while the germination percentage of seedlings treated with 20% dilution was 96% in comparison to control. At high concentration of treatment levels 40%, 60%, 80% and 100% the germination percentage were decreased 70%, 56%, 40% and 33% respectively. The maximum decrease was found at 100% (33%) effluent concentration.

Vigour index in untreated seedling (control) of *Glycine max L.* was 1590.3 while the vigour index of seedlings treated with 20% dilution was increased 1680 in comparison to control. At high concentration of treatment levels 40%, 60%, 80% and 100% the vigour index were decreased 1204, 856.8, 436 and 234.3 respectively. The maximum reduction was found 85.26% at 100% effluent concentration.

Root length in untreated seedling (control) of *Glycine max L.* was 4.5cm while the root length of seedlings treated with 20% and 40% dilution were increased 4.7 and 4.6 cm in comparison to control. At high concentration of treatment levels 60%, 80% and 100% the root length were decreased 3.4, 2.1 and 1.9 cm respectively. The maximum reduction was found 57.7% at 100% effluent concentration.

Shoot length in untreated seedling (control) of *Glycine max L.* was 12.6 cm while the root length of seedlings treated with 20% and 40% dilution were increased 12.8 cm and no change at 40% in comparison to control. At high concentration of treatment levels 60%, 80% and 100% the shoot length were decreased 3.4, 2.1 and 1.9 cm respectively. The maximum reduction was found 57.7% at 100% effluent concentration.

Fresh weight in untreated seedling (control) of *Glycine max* was 3.10 gm while the fresh weight of seedlings treated with 20% and 40% dilution were increased 3.26 and 3.15 gm in comparison to control. At high concentration of treatment levels 60%, 80% and 100% the fresh weight were decreased 2.98, 2.42 and 2.10 gm respectively. The maximum reduction was found 32.25% at 100% effluent concentration.

Dry weight in untreated seedling (control) of *Glycine max L.* was 1.90 gm while the dry weight of seedlings treated with 20% and 40% dilution were increased 1.98 and 1.93 gm in comparison to control. At high concentration of treatment levels 60%, 80% and 100% the dry weight were decreased 1.51, 1.26 and 1.09 gm respectively. The maximum reduction was found 42.63% at 100% effluent concentration.

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Chlorophyll a content in untreated seedling (control) of *Glycine max L.* was 5.06 mg/gm while the Chlorophyll a of seedlings treated with 20% and 40% dilution were increased 5.11 and 5.08 mg/gm in comparison to control. At high concentration of treatment levels 60%, 80% and 100% the Chlorophyll a were decreased 4.86, 3.87 and 2.42 mg/gm respectively. The maximum reduction was found 52.1% at 100% effluent concentration.

Chlorophyll b content in untreated seedling (control) of *Glycine max L.* was 5.02 mg/gm while the Chlorophyll b of seedlings treated with 20% and 40% dilution were increased 5.07 and 5.05 mg/gm in comparison to control. At high concentration of treatment levels 60%, 80% and 100% the Chlorophyll b were decreased 3.71, 2.69 and 1.80 mg/gm respectively. The maximum reduction was found 64.1% at 100% effluent concentration.

Present study result revealed that effluent of dying industries inhibit seed germination and seedling growth. It may be due to the presence of toxic elements and metal ions in the corresponding industrial effluents (Rohit et al. 2013). The higher pH, BOD, COD and other higher organic loads that cause adverse effect on germination (Panday et al. 2004 and Saddaqat et al. 2006. This could be related to the fact that some of the nutrients present in the effluent are essential but in higher concentration they become hazardous and toxic to the soybean plant (Ravi et al. 2014). The exposure of lower concentration of effluent to the seedling shows growth promotion, over all development of the seedling and chlorophyll content. Reduction in seed germination percentage at higher concentration of effluent may be due to the higher amount of solids presents in the effluent, which causes in the osmotic relationship of the seed and water.

Present study supported with the views of Sundaramoorthy et al. (2000) who investigated that the percentage of seed

germination and seedling growth was maximum at 10% diluted effluent than the control while undiluted effluent showed inhibitory effects . Hussain et al. (2013) also reported that diluted effluent (25%) increase the growth parameters and pigments in the Maize seedlings. Kathirvel (2012) also supported present study who investigated that at 20% concentration of effluent, the plant showed maximum germination, root and shoot length than the control and the maximum chlorophyll content were also reported at 20% effluent concentration which could be due to the best growth of seedling at this concentration. Present study is also supported by Mayuri et al. (2015) who reported that the best germination and seedling growth was observed in 25% concentration with growth promoting effect and significantly better than control. Beyond 25% effluent germination percentage and seedling growth decreased gradually. Divyaprivas et al. (2014) also concluded that germination percentage and other biochemical contents were high at 30% effluent dilution in comparable with control but beyond 30% dilution all parameters were decreased and at 100% effluent treatment the seed germination was completely inhibited in *Cicer arietinum*.

4. Conclusion

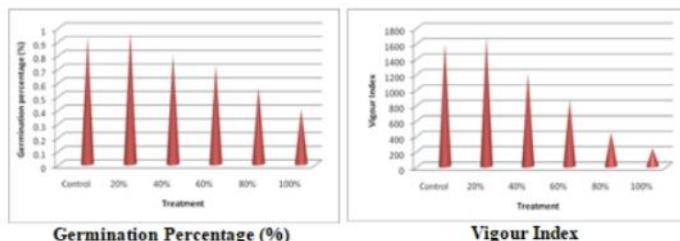
The present study concluded that the dying effluent waste significantly influence growth parameters of soybean (*Glycine max L.*). The collected effluent sample contains anion which can be beneficial for plant growth but its excessive level could be toxic, retard the growth of the plants. Reduction in seed germination percentage at higher concentration of effluent may be due to the higher amount of solids present in the effluent, which causes changes in the osmotic relationship of the seed and water. The results suggested that dye industrial effluent (20%) could be used for irrigation of soybean crops.

Table 1: Effect of dye effluent on some growth parameters of *Glycine max L*

S. No.	Treatment	Seed germination Percentage (7th days)	Vigour index	Seedling growth (14 th days)		Weight		Chlorophyll Content (mg/gm)	
				Shoot length (cm.) Avg.	Root length (cm) Avg.	Fresh weight (gm)	Dry weight (gm)	Chlorophyll a (mg/gm)	Chlorophyll b (mg/gm)
1.	Control	93	1590.3	12.6	4.5	3.10	1.90	5.06	5.02
2.	20%	96	1680(5.4%)*	12.8 (1.5%)*	4.7(4.4%)*	3.26(5.1%)*	1.98(9.2%)*	5.11(0.98%)*	5.07(0.99%)*
3.	40%	70	1204(24.2%)*	12.6	4.6(2.2%)*	3.15(1.5%)*	1.93(1.5%)*	5.08(0.39%)*	5.05(0.59%)*
4.	60%	56	856.8(46.1%)*	11.9 (5.5%)*	3.4(24.4%)*	2.98(3.8%)*	1.51(20.5%)*	4.86(3.95%)*	3.71(26.09%)*
5.	80%	40	436(72.5%)*	8.8 (30.1%)*	2.1(53.3%)*	2.42(21.9%)*	1.26(33.6%)*	3.87(23.5%)*	2.69(46.4%)*
6.	100%	33	234.3(85.2%)*	5.2 (58.7%)*	1.9(57.7%)*	2.10(32.2%)*	1.09(42.6%)*	2.42(52.1%)*	1.80(64.1%)*

* Figures in parenthesis showed decrease over control.

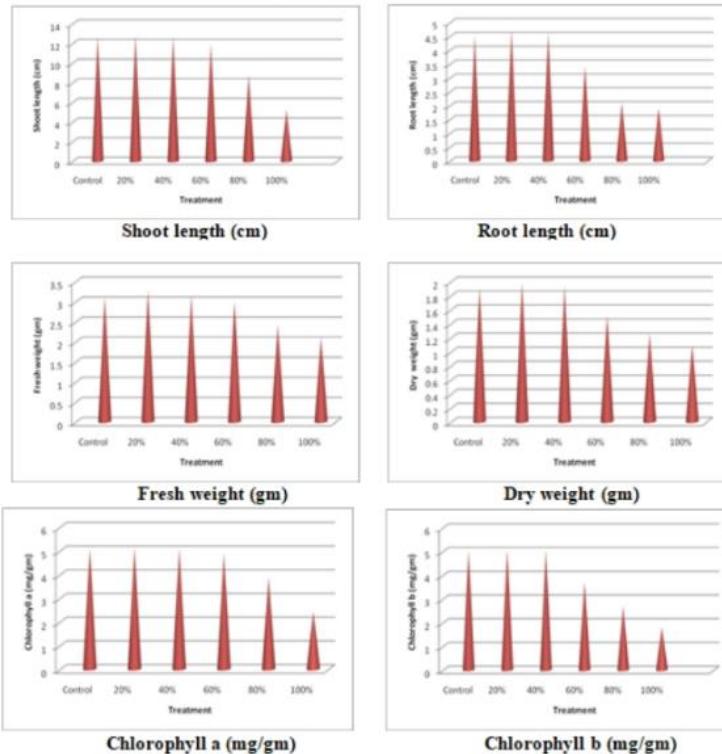
** Figures in parenthesis showed increase over control



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Graph showing impact of dyeing and printing effluent on seed germination, seedling growth and chlorophyll content of Soybean (*Glycine max* L.).



Figure: 3rd day germination state in *Glycine max*



Figure 7: 14th day root length of *Glycine max*

References

- [1] Abdul Baki A.A. and Anderson, J.D. Vigour determination of soybean seeds by multiply criteria. *Crop sci.*, 1973. Vol. 13: 630-633.
- [2] Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* Vol.24: 1-15.
- [3] Correia V. M, T. Stephenson, SJ Judd, 1994. Characterization of textile wastewater- a review. *Environmental Technology*. Vol.15: 917-929.
- [4] Hussain I., M. Iqbal, M. Nawaz, R. Rasheed, A. Perveen, S. Mahmood, A. Yasmeen and A. Wahid, 2013. Effect of sugar mill effluent on growth and Antioxidative Potential of Maize Seedling. *International Journal of Agriculture & Biology*. Vol.15(6): 1227-1235.
- [5] Rehman S., Harris P.J.C. and Bourne W.P., 1998. Effect of pre sowing treatment with calcium salts. *Acacia seeds, J. Plant nutrition*, Vol. 21: 277-285.
- [6] Kathirval P., 2011. The effect of dye effluent on growth, yield and biochemical attributes of Bengal Gram (*Cicer arietinum* L.). *International Journal of Applied Biology and Pharmaceutical Technology*. Vol.3(1): 146-150.

International Journal of Science and Research (IJSR)

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- [7] Kaushik A., B.R.Kadyan and C.P.Kaushik., 2004. Sugar mill effluent effects on growth, photosynthesis pigments and nutrients uptake in wheat seedlings in aqueous vs. soil medium. Water Air soil poll. Vol.87: 36-46.
- [8] Nail D.J., K.K. Desai and R.J. Vansi, 2009. Physico-chemical characterization of chemical sludge generated from treatment of combined waste water of dyes and dye intermediate manufacturing industries. J. Environ. Res. Develop. Vol.4(2): 413-416.
- [9] Nath et al., 2007. Combinatorial effects of distillery and sugar factory effluents in crop plants. Journal of Environmental Biology. Vol.28: 577-582.
- [10] Online at : file://H/Soybean- Wikipedia, the free encyclopedia.html.
- [11] Panday S.N, 2004. Industrial effluent on seed germination and seedling growth of *Zea mays* Linn. And *Oryza sativa* Linn. Biological memories. Vol.30: 104-107.
- [12] Parameswari M., 2014. Effect of textile and dye effluent irrigation on germination and its growth parameters of Green Gram, Black Gram and Red Gram. International Journal of Environmental Science and Technology Research. Vol.2(1): 6-10.
- [13] Patel H. and S. Panday, 2008. Physico-chemical characterization of textile chemical sludge generated from various CETPS in India. J. Environ. Res. Develop. Vol.2(3): 229-239.
- [14] Prabhakar P.S., M. Mall and J. Singh, 2006. Impact of fertilizer factory effluent on seed germination, seedling growth and chlorophyll content of gram (*Cicer arietinum*). Journal of Environment Biology. Vol.27(1): 153-156.
- [15] Ravi D., R. Parathasarathy, V. Vijayabharathi and S. Suresh, 2014. Effect of textile dye effluent on Soybean crop. Journal of Pharmaceutical, chemical and biological sciences. Vol.2(2): 111-117.
- [16] Rohit K.C. and P. Ponmurgan, 2013. Seed germination study of *Vigna radiata* using treated and untreated industrial effluents. International Journal of Latest Research in Science and Technology. Vol.2(2): 103-104.
- [17] Saddaqat Ali, R. Nadeem, H. Nawaz, S. Hayat, S. Ali and M. Muneer, 2006. Analyses and treatment of textile effluent. International Journal of Agriculture and Biology. Vol.8(5): 641-644.
- [18] Saxena R.M., P.F. Kewa, R.S. Yadav and A.K. Bhatnagar, 1986. Impact of tannery effluent on some pulse crop. Indian J. Environ. Hlth. Vol.28(4): 345-348.
- [19] Singh K.P. et al., 2004. Impact assessment of treated/untreated wastewater toxicants discharged by sewage treatment plants on health, agricultural, and environmental quality in the wastewater disposal area. Chemosphere. Vol.55: 227-255.
- [20] Sundaramoorthy P., S. Saravanan, A. Subraman and A.S. Laashmanachary, 2000. Toxicity effect of fertilizer factory effluent on seed germination and seedling growth of some agriculture crops. Poll. Res. Vol.19(4): 529-533.

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Impact of dye effluent on growth and chlorophyll content of Alfalfa (*Medicago sativa* L.)

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Abstract: The textile industry plays an important role in the world economy as well as in our daily life time, it consumes large quantity of water and generates huge amount of waste water. The chemical reagents used in the textile sector are diverse in chemical composition ranging from inorganic to organic molecules. The presence of these chemicals will show detrimental effects on the germination process and growth of seedlings. Present research work has been carried out to study the impact of effluent at different concentrations (20%, 40%, 60%, 80%, 100%) on seed germination and seedling growth of *Medicago sativa*. On the 14th day of seedling growth, maximum root and shoot length were observed at 20% concentration of effluent i.e. 5.4cm (root length) and 5.1cm (shoot length) which increased 3.8% and 4.0% respectively in comparison to control. At high concentrations of treatment levels root length was decreased 2.04%, 19.2%, 26.5%, 51.0% respectively and shoot length was decreased 3.84%, 17.3%, 26.9%, 44.2% respectively at 40%, 60%, 80% and 100% treatment levels in comparison to control (4.9cm and 5.2cm respectively). Same trend was observed during estimation of dry weight and chlorophyll contents. Inhibition of seedling germination and seedling growth at higher concentrations of effluent may be due to high level of dissolved solids which enhance the salinity. The present study concluded that the dyeing effluent waste significantly influence growth parameters of *Medicago sativa*.

Key words: *Medicago sativa*; textile dye effluent; germination; seedling growth; chlorophyll contents

Introduction

Dying and printing industries are one of the most water consuming industries, responsible for water and soil pollution. These industries constantly adding up toxic substances in to the ground water reservoir in the industrial zone and used for irrigation. These toxic substances can be transferred and get accumulated into plant tissues from soil and have damaging effects on plant themselves and may become a health problem to man and animals (Garg et al., 2007). Wynne et al., (2001) noted that textile effluent is highly colored and saline contain non-biodegradable compounds and are high in biological oxygen demand (BOD), chemical oxygen demand (COD), sodium and other dissolved solids as well as micronutrients and heavy metals (Martin et al., 1994). These effluents cause coloration of water when released untreated into the water bodies and cause severe problems to aquatic life (Hai et al., 2007). The use of such waste water in irrigation system definitely provides some nutrients to enhance the fertility of soil properties in the long run (Yasmin et al., 2011). Accumulation of excessive salts makes the soil saline, while presences of excessive color in effluent pollutes the water bodies and prevent the penetration of light, which in turn impedes with the photosynthetic activities of aquatic flora (Ranganathan and Kurian, 1997). Many scientists

have documented adverse effects of different industrial effluents on the growth of plants and dye waste water has also been found toxic to several crop plants (Kaushik et al., 2005, Sundaramoorthy et al., 2001). Malaviya et al., (2012) demonstrated that at lower concentration of dying and industrial effluent cause a positive impact on germination and growth of *Pisum sativum*. At 100% effluent concentration nutrients were raised to high to become toxic resulting in retarded root and shoot length. Germination of seed is a critical stage which insures reproduction and consequently the control of dynamic population to it at least for proper crop productivity. Hence considering all the good and bad effects of industrial effluents on crop plants, the present study was conducted by using effluents obtained from dying and printing industry as a source of water to germinate seedling of *Medicago sativa* and its effect was observed on growth and chlorophyll content.

Materials and Methods

Experimental Plant

Alfalfa (*Medicago sativa* L.) is the most cultivated forage legume in the world due to its high nutritional quality, high protein content and effects on soil fertility. It has played an important role as a livestock forage. The medicinal uses of alfalfa stem

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from anecdotal reports that the leaves causes diuresis and are useful in the treatment of kidney, bladder and prostate disorders. Its extracts are used in baked goods, beverages, and prepared foods and the plant serves as a commercial source of chlorophyll.

Collection of Effluent sample

The effluent samples were collected from small dying and printing units from kaithun region, Kota, Rajasthan. Samples were collected in sterilized wide mouth poly ethylene bottles and were stored at 4°C temperature to avoid any changes on its characteristics. To evaluate the effects of dyeing effluent on *Medicago sativa*, solution of different concentrations of effluent were prepared.

Treatment Level

C- Control (100% Distilled water)
 T₁- Effluent: Distilled water (20% + 80%)
 T₂- Effluent: Distilled water (40% + 60%)
 T₃- Effluent: Distilled water (60% + 40%)
 T₄- Effluent: Distilled water (80% + 20%)
 T₅- Effluent (100%)

For germination tests, seeds were sterilized with 0.1% w/v mercuric chloride (HgCl₂) solution for 5 minutes to remove microbes and then washed three times with sterile distilled water. 20 seeds of Alfalfa were placed in sterilized glass petri dishes of uniform size lined with filter paper discs. These filter discs were then moistened with 5 ml of distilled water for control and with the same quantity of various concentrations of the textile effluent (20%, 40%, 60%, 80%, 100%). The Petri dishes were incubated at room temperature. 2 ml of respective dilutions were sprayed for consecutive 6 days. Germination was recorded daily. All the levels were carried out in triplicate. On the 7th day, germination percentage was calculated and various growth parameters and chlorophyll content were evaluated on 14th day of the experiment.

Germination percentage

The formula given by Rehman *et al.*, (1998) was used to estimate germination percentage.

$$\text{Germination percentage} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds}} \times 100$$

Root and Shoot length

Length of root and shoot of seedlings were calculated by using the standard centimeter scale.

Vigour index

The formula suggested by Abdul-Baki and Anderson (1973) was used to calculate vigour index.
 Vigour index = germination percentage² (root length^{*} + shoot length^{*})
 (* indicate that length of root and shoot in cm.)

Fresh and Dry Weight of seeds

Ten seeds of each treatment were weighted in order to determine the fresh weight and then dried in oven at 80°C for 24 hrs. to obtain dry weight. Fresh weight and Dry weight were recorded in gms.

Chlorophyll estimation

The estimation of chlorophyll content was done according to Arnon's method (1949).

$$\text{Chlorophyll a (mg/g)} = \frac{12.7 A_{663} - 2.69 A_{645}}{A \times 1000 \times W}$$

$$\text{Chlorophyll B (mg/g)} = \frac{22.9 A_{645} - 4.68 A_{663}}{A \times 1000 \times W}$$

Where

A₆₆₃= Absorbance at wavelength 663 nm

A₆₄₅= Absorbance at wavelength 645 nm

V= Volume of the extract in ml

A = Length of light path in the cuvette (1cm)

W= Fresh weight of the samples in gm.

Result and Discussion

The present study was carried out to assess the effect of dying industry effluent on germination and seedlings growth to determine the tolerance level of Alfalfa (*Medicago sativa*). The result revealed that *Medicago sativa* shows positive growth response to the dying effluent at treatment level T₁ (20% Effluent+ 80% D/w). Seedling growth showed increase in shoot and root lengths at T₁ whereas a gradual decline was observed in growth parameters with the increasing concentration of dying effluent at levels T₂-T₅ (40%, 60%, 80%, 100%). In the present study at 20% concentration of effluent the seedling growth showed maximum root length 5.4cm and shoot length 5.1cm i.e. 3.8% and 4.0% in comparison to control but at high concentrations of treatment level (40%, 60%, 80%, 100%) root length was decreased 2.04%, 19.2%, 26.5%, 51.0% respectively and shoot length was decreased 3.84%, 17.3%, 26.9%, 44.2% respectively in comparison to control. Other parameters were also decreased at higher concentrations of effluent. Values of various parameters for different concentrations of the effluent are given in table 1.

Table 1: Effect of different concentrations of effluent on various parameters of *Medicago sativa*

S.No.	Treatment	Seed germination Percentage (7 th Days)	Vigour Index	Seedling Growth (14 th Days)		Weight		Chlorophyll content (mg/gm)	
				Shoot length (cm)	Root length (cm)	Fresh weight (gm)	Dry weight (gm)	Chl a (mg/gm)	Chl b (mg/gm)
1.	Control	83%	945	4.9	5.2	1.57	0.51	3.02	2.96
2.	20%	90%	(12.7%)**	(4.0%)**	(3.8%)**	(12.7%)**	(11.7%)**	(6.2%)**	(1.6%)**
3.	40%	81%	(5.3%)*	(2.04%)*	(3.84%)*	(3.18%)*	(3.92%)*	(14.5%)*	(5.40%)*
4.	60%	66%	(33.8%)*	(19.2%)*	(17.3%)*	(11.4%)*	(17.6%)*	(32.7%)*	(13.5%)*
5.	80%	50%	(55.8%)*	(26.5%)*	(26.9%)*	(39.4%)*	(31.3%)*	(36.4%)*	(39.1%)*
6.	100%	33%	(79.1%)*	(51.0%)*	(44.2%)*	(66.8%)*	(56.8%)*	(66.5%)*	(64.5%)*

*Figures in parentheses represent % decrease over control.

**Figures in parentheses represent % increase over control.

From the experimental data it has been revealed that in *Medicago sativa* suppression of germination, shoot and root lengths and chlorophyll contents (chl.a and chl.b) were affected at higher concentration (above 40%) of effluent may be due to high levels of total dissolved solids which enhance the salinity and conductivity of the solute absorbed by the seeds before germination. Our findings are also in accordance with the outcome obtained by Wins *et al.*, (2010) who investigated that at 25% concentration along with the growth promoting effect, significantly better than control. Beyond 25% effluent, root and shoot length decreased. Similar results were made by Lenin *et al.*, (2014) who reported that the best germination of seedling growth, root length, shoot length, fresh weight and dry weight and tolerant variety were observed with 20% effluent concentration of sago factory effluent with growth promoting effect significantly better than control. Beyond 20% effluent concentration, root and shoot length were reportedly decreased. The same trend was observed by Mohammad and Khan (1985) while studying the effect of textile industry effluent and they found that no adverse effect of textile industry effluent at lower concentration (< 50% effluent concentration) which is in conformity with the present results. Present study also supports the view of Chinnusamy *et al.*, (2001) who observed that root length, shoot length, fresh weight root and shoot, dry weight of root and shoot, germination relative index, vigour index and chlorophyll content were higher in 25% than 50% over control. It also supported by Parameswari M. (2014) who reported that concentrated textile and dyeing effluent adversely affect the crop plants, although diluted textile and dye effluent with water in 1:3 ratio did not have any adverse effect on the growth and vigour index of field crops and the diluted effluent increased the germination and vigour index of groundnut.

In this paper an attempt has been made to assess the effect of textile and dyeing effluent on germination and seedling growth of Alfalfa

(*Medicago sativa*). Present study concluded that seed germination percentage, vigour index, root and shoot length, fresh and dry weight and chlorophyll content were higher in lower effluent concentration (20% effluent) but decrease with the increasing concentration of effluent (40%, 60%, 80%, 100%).

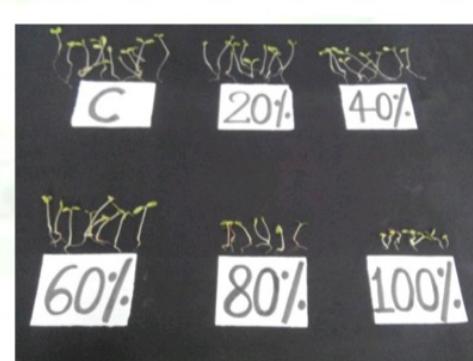
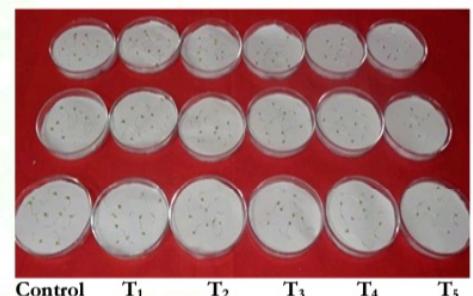


Fig. 14th day germination state in *Medicago sativa* L.

References

- Abdul Baki A.A. and Anderson, J.D. Vigour determination of soybean seeds by multiply criterias. *Crop sci.*, 1973. Vol. 13: 630-633.
- Arnon, D.I. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.*, 1949. Vol. 24: 1-15.

Pratibha and Azra Akhtar,

Annals of Plant Sciences 5.10 (2016): 1432-1435

3. Chinnusamy, Annadurai C. K., Jayanthi C., Veeraputhriyan R. and Karunanthi S. Organic amendments and distillery effluent on soil fertility and productivity of rice. Proceedings of National Seminar on Use of Poor Quality Water and Sugar Industrial Effluents in Agriculture, 2001 Feb. 5, TNAU, Tiruchirappalli, 84-84.
4. Garg V.K., Kaushik P. Influence of textile mill waste water irrigation on the growth of Sorghum cultivars. Applied ecology and environmental research, 2007, 6(2), 1-12.
5. Hai, F.I., K. Yamamoto and K. Fukushi. Hybrid treatment systems for dye wastewater. Critical Rev. Environ. Sci. Technol., 2007, 37, 315-377.
6. Kaushik P., Garg V.K., Singh B. Effect of textile effluent on different cultivar of wheat. *Biores. Technol.*, 2005, 96, 1189-1193.
7. Lenin M., Mariyappan K.S. and Thamarikannan M.R. Effect of sago factory effluent on seed germination and seedling growth of gingelly (*Sesamum indicum* L.) varieties. Int. J. Life Sc. Bt. & Pharm. Res., 2014, 3(1), 2250-3137.
8. Malaviya P., Rajesh H., Neeru S. Impact of dyeing industry effluent on germination and growth of pea (*Pisum sativum*). J. Environ. Biol., 2012, 33, 1075-1078.
9. Martin M.H. and Bullock R.J. The impact and fate of heavy metals in an Oak woodland ecosystem. In: M. Ross(ed): Toxic metals in soil-plant system, 1994. John Wiley & Sons, New York. 327-363.
10. Mohammad A., Khan AA.U.. Effect of textile factory effluent on soil and crop plants. Environ. Pollut., 1985. (Series A), 37, 131-148.
11. Parameswari M. Effect of textile and dye effluent irrigation on germination and its growth parameters of green gram, black gram and red gram. International journal of environmental science and toxicology research 2014, 2(1), 6-10.
12. Ranganathan KM, Kurian J. Industrial effluent management for clusters of textile bleaching and dyeing units. Proc. 6th Natl. Symp. On Environ., 1997 Coimbatore, 84-88.
13. Rehman S., Harris P.J.C. and Bourne W.P. Effect of pre-sowing treatment with calcium salts. Acacia seeds, *J. Plant nutrition*, 1998, 21, 277-285.
14. Sundaramoorthy P., Kunchithapatham J., Thamizhiniyan P. and Venkateslu V. Effect of fertilizer factory effluent on germination and seedling growth of groundnut varieties. *J. Ecobiol.*, 2001, 13(1), 03-08.
15. Wins. J.A. And Murugan M. Effect of textile mill effluent on growth and germination of Black gram *Vigna mungo* (L.) Hepper. *Int. J. Pharma. Biosci.*, 2010, 1, 1-7.
16. Wynne G., Maharaj D. and Buckley C. Cleaner production in the textile industry. Lessons from the Danish experience, school of chemical engineering, 2001. University of Natal, Durban, South Africa, 3.
17. Yasmin A., Nawaz S and Ali S. M. Impact of industrial effluents on germination and seedling growth of *Lens Esculentum* varieties. Pak. J. Bot., 2011, 43(6), 2759-2763.

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Research Article

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Physico-Chemical Characterization of Soil and Effluent of Dye Industries in Kaithun region of Kota, Rajasthan

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ABSTRACT

Industrial Effluent entering the water bodies is one of major source of environmental toxicity. It not only affects the quality of drinking water but also has deleterious impact on the soil. Industries keep on releasing effluents, which are quite toxic. Soil is the most favorable habitat for a wide range of microorganisms that includes bacteria, fungi, algae and viruses. In the present study was an attempt to assess quality of polluted soil and the physico-chemical properties of dye industries effluent of Kaithun Region, Kota.

Keywords: Dye industrial effluent, contaminated soil, physico-chemical analysis.

INTRODUCTION

Nature has an amazing ability to cope up with small amount of water wastes and pollution, but it would be hazardous if billions of gallons of waste water produced everyday are not treated before releasing them back to the environment. The quantities and characteristics of discharged effluent vary from industry to industry depending on the water consumption and average daily product. Dye effluent mainly contains 1.) Large amount of total dissolved solids limiting the industrial and agricultural use of water 2.) High levels of chemical oxygen demand (indicating high degree of pollution) and biological oxygen demand⁶. Soil is also one of the vital resources on living planet Earth. It is heterogeneous in nature. Many scientists have documented adverse effects of different industrial effluents on the growth of plants and dye waste water has also been found toxic to several crop plants⁴.

One of the main sources with severe pollution problems worldwide is the textile industries and its dye-containing wastewater. 10-25% of textile dyes are lost during the dyeing process and 2-20% is discharged as aqueous effluents in different environmental components¹. Waste water from any industries is often rich in color, containing residues of reactive dyes, organic chemicals and bleaching agents. Heavy metals like zinc, copper, manganese, iron etc are present in the dye effluents. The present investigation was aimed to know the effect of dye industrial effluent on water and soil quality. It have been reported that the major problem associated with textile processing effluents is presence of heavy metal ions, which arise from material used in the dyeing process or in a considerably high amount, from metal containing dye. Most of the heavy metals are essential for growth of organisms but are only required in low concentrations.

The present study was carried out to characteristic dyeing industry effluent in turn to their physico-chemical properties and to evaluate the impact of industrial effluent on the soil near small dyeing industries in Kaithun region near Kota (Rajasthan).

MATERIALS AND METHODS

Collection of Effluent Samples:

The sampling was carried out in October 2013. The sampling site in the present paper is a local textile mill (STM) located near Kota city in Rajasthan. Samples were collected from the 2 different sites of the selected area in a wide mouth plastic bottle. Reference water sample was taken from hand pump 1 km away from the mill area and tap water 2 km away from the mill area.

Collection of Soil Samples:

The soil samples were collected from 0-30 cm depth from three locations immediate to dumping site of effluent printing cluster for the study. The collected soil samples have been analyzed for physicochemical parameters like pH, organic carbon, electric conductivity, nitrogen, calcium, magnesium, potassium, phosphorus, water holding capacity and heavy metals like Zn, Cu, Mn, Fe.

Methods:

1. Physico-chemical analysis of the textile industry effluents-

The site of sample collection was identified at point where the effluent is discharged from the mill. The color of the effluent and smell was observed at the time of collection of the sample in sterile bottles. The wastewater discharged from the textile industries is characterized by a variety of chemicals generated from dyeing and washing processes. It also constitutes suspended solids, organic and inorganic matters, acid and alkalies. Textile wastewater contains substantial pollution loads in terms of BOD, COD, TSS and heavy metals. The environmental concern of discharged textile wastewater is mainly its high chemical oxygen demand (COD) as well as high strength of color content. The analysis were carried out as per the standard methods².

2. Physico-chemical analysis of the soil contaminated by textile industrial effluent-

The soil samples were collected from the experimental site where untreated effluent was discharged by the industry. Three replicates of each samples from three different sites (S₁, S₂, S₃) were collected from 0-30 cm. depth from various locations. The homogenized samples were air dried for seven days, gently crushed with a wooden roller and passed through 2 mm sieve. All samples were analyzed by carried out as per the standard methods.

The physico-chemical parameters like pH, Ec, BOD, COD, TDS, Hardness, organic carbon, Water holding capacity, N, P, K and Heavy metals etc assessed in the dyeing effluent and polluted soil were higher than the recommended standard for discharge of industrial effluent by BIS.

RESULT AND DISCUSSION

Physico-chemical characterization of the textile dye effluent samples were analyzed for different Physico-chemical parameters as shown in Table 1. It showed that effluent have dark blue and black color with pungent smell, relatively high temperature 45° (measured by a laboratory thermometer). The pH of the effluents was slightly to moderate basic range ranged from 7.2-7.9.

Electrical Conductivity of effluent samples ranged from 1470-2200 $\mu\text{S}/\text{cm}$. Biological Oxygen Demand was ranged from 488-1090 mg/l and Chemical Oxygen Demand was ranged from 3120-6864 mg/l. Total Dissolved Solids of effluent ranged from 980-1440 mg/l. Calcium hardness ranged between 110-800 mg/l and Magnesium hardness ranged between 120-1450 mg/l. Total hardness ranged from 230-2250 mg/l. Oil and Grease in effluent samples ranged from 21-43.6 mg/l.

The soil samples adjoining the textile effluent, of mill region also show great variation in the physico-chemical properties. The pH of the soil samples ranged from 7.9-8.1. Electrical conductivity ranged from 0.21-0.44 ds/m. Organic carbon (%) of soil samples between 0.53-0.96%. Values of nitrogen (N), phosphate (P) and potash (K) concentration in the soil samples were also shows to have great variability. NPK concentration of soil samples exhibited that N, P, and K concentration ranged from: N (2.81-6.22 ppm), P (16.6-69.3 ppm), K (314-653 ppm). Calcium and Magnesium of soil samples ranged from 3.0-3.6 ppm and 3.4-4.0 ppm. Water holding capacity of soil samples ranged between 45.27-45.86%.

Mahawar, P. and Akhtar, A. *Int. J. Pure App. Biosci.* **3 (2)**: 419-422 (2015) ISSN: 2320 – 7051
 Heavy metals such as Zn, Cu, Mn, Fe ranged from: Zn (0.3-6.8 mg/g), Cu (0.8-48.65mg/g), Mn (4.3-11.5mg/g) and Fe (0.2-9.0mg/g).

Table -1: Physico-chemical characterization of Dye effluent

S/N	PARAMETERS	EFFLUENT SAMPLE		CONTROL SAMPLE	
		S1	S2	C1	C2
1.	pH	7.20	7.97	8.2	8.3
2.	Biological Oxygen Demand (mg/l)	1090	488	0.4	1.0
3.	Chemical Oxygen Demand (mg/l)	6864	3120	64	52
4.	Oil and Grease (mg/l)	43.6	21	-	-
5.	Electrical Conductivity (umho/cm ²)	1470	2200	1148	1212
6.	Total Dissolved Solides (mg/l)	980	1440	861	909
7.	Calcium Hardness	800	110	160	180
8.	Magnesium Hardness	1450	120	130	130
9.	Total Hardness	2250	230	290	310
10.	Color	Dark blue	Black	Colorless	Colorless

Table -1: Physico-chemical characterization of soil contaminated by Dye effluent

S.N.	Sample	Parameters												
		pH	EC	%OC	Na	P	K	Ca	Mg	%WHC	Zn	Cu	Mn	Fe
1.	S1													
	10 cm.	7.99	0.21	0.95	618	44	653	3.2	3.6	45.27	6.886	48.65	11.30	0.253
	20 cm.	8.13	0.21	0.93	600	16.2	469	3.6	4.0	45.30	6.606	47.43	11.50	1.078
	30 cm.	8.13	0.22	0.96	622	69.3	389	3.4	3.7	44.38	5.404	48.56	11.27	3.094
2.	S2													
	10 cm.	8.00	0.44	0.74	499	16.6	597	3.1	3.5	45.86	0.778	9.372	9.353	9.02
	20 cm.	8.06	0.30	0.86	541	49.5	408	3.3	3.6	45.79	0.359	4.774	8.040	3.583
	30 cm.	8.06	0.29	0.80	535	49.9	314	3.0	3.4	43.98	0.339	3.145	8.257	3.703
3.	S3													
	10 cm.	8.05	0.31	0.77	509	17.5	501	3.5	4.0	43.41	2.093	0.887	5.438	5.08
	20 cm.	8.19	0.26	0.57	281	32.7	407	2.9	3.4	41.52	1.584	0.976	4.318	5.141
	30 cm.	8.17	0.23	0.53	256	18.8	325	3.4	3.8	37.91	1.625	1.103	5.631	4.316

CONCLUSION

This study has shown that KTM (kaithun Textile Mills), is well known for using eco friendly natural dyes for dyeing and hand block painting, thus the untreated textile effluent should be less polluted. But the results show the different case as some parameters like TDS, BOD, COD etc. exceeds the WHO limits at significant level. Also the concentration of heavy metals was found to be high which might be due to the use of mordents and synthetic dyes. The result indicating that the application of textile effluent/polluted water affect physico-chemical properties of soil. This study also reveals that effluent from KTM was highly polluted, there is urgent need to follow effluent treatment methods before their discharge to surface water for reducing their potential environmental hazards.

REFERENCES

- Ahmad, M.M. Sushil and M, Krishna. Influence of dye industrial effluent on physico chemical characteristics properties of soil at Bhairavgarh, Ujjain, MP, India. *I Research Journal of Environment Sciences*, **1(1)** : 50-53 (2012)

- Mahawar, P. and Akhtar, A.** *Int. J. Pure App. Biosci.* **3** (2): 419-422 (2015) ISSN: 2320 – 7051
2. APHA, Standard methods for the examination of water and waste water 20th addition. Washington, D.C: American Public Health Association, WPCF and AWWA (1998)
 3. Esabela, Sharma, K.C. and Chauhan, S.S. Physico-chemical profile of untreated irrigation water from Amanishah Nalla, Sanganer, (Jaipur). *An International Quarterly of Environmental Sciences*, **5(1&2)**: 55-58 (2011)
 4. Joshi, N. and Kumar, A. Physico-chemical analysis of Soil and Industrial Effluents of Sanganer. *Research Journal of Agricultural Sciences*, **2(2)**: 354-356 (2011)
 5. Jolly, Y.N. Islam, A. and Mustafa, A.I. Impact of Dyeing Industry Effluent on Soil and Crop. *Universal Journal of Environmental Research and Technology*, **2(6)**:560-568 (2012)
 6. Joshi, V.J. and Santani, D.D. Physicochemical Characterization and Heavy Metal Concentration in Effluent of Textile Industry. *Universal Journal of Environmental Research and Technology*, **2(2)**: 93-96 (2012)
 7. K.C., Rohit and Ponmurugan, P. Physico-chemical analysis of textile, automobile and pharmaceutical industrial effluents. *International Journal of Latest Research in Science and Technology*, **2(2)**:115-117 (2013)
 8. Kaur, A. Vats, S. Rekhi, S. Bhardwaj, A. Goel, J. Tanwar, R. Gaur, K. Physico-chemical analysis of the industrial effluents and their impact on the soil microflora. *International Society for Environmental Information Sciences*, **2**: 595-599 (2010)
 9. Mehta, R. and Yadav, K. Soil contamination due to textile effluent- Case study on the Printing cluster of Jaipur. *Textile Association, Dept. of Clothing & Textile, IIS University, Jaipur* (2013)
 10. Rathore, J. Studies on pollution load induced by dyeing and printing units in River Bandi at Pali, Rajasthan, India. *International Journal of Environment Science*, **3(1)** (2012)
 11. Samuel, S. and Muthukkaruppan, S.M. Physico-chemical analysis of Sugar Mill Effluent, Contaminated Soil and its Effect on Seed Germination of Paddy (*Oryza sativa L.*). *International Journal of Pharmaceutical & Biological Archives*, **2(5)**: 1469-1472 (2011)
 12. Thoker, A.F. M, Sushil and M, Krishna. Impact of Dye Industrial Effluent on Physicochemical Characteristics of Kshipra River, Ujjain City, India. *International Research Journal of Environment Sciences*, **1(2)**: 41-45 (2012)
 13. Versa, G. Sudesh and Singh, S. Physico-chemical analysis of textile effluents of Dye and Printing clusters of Bagru region, Jaipur, India. *Journal of Environmental Research And Development*, **8(1)**: (2013)
 14. Wokhe, T.B. Mohammed, Yahaya and Paschal Chima, Madu. Evaluation of Physicochemical properties of Irrigated Soil. *Journal of Natural Science Research*, **3(9)** (2013)



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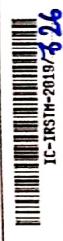
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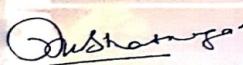
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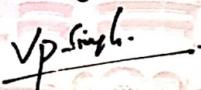
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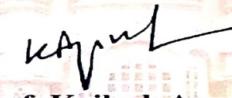


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