Treatment of industrial dye waste water and its effect on growth of *Pisum sativum*

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Submitted by

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CERTIFICATE

This is to certify that the M. Tech. dissertation entitled **"Treatment of industrial dye waste water and its effect on growth of *Pisum sativum*"**, submitted by **K.LAVANYA (2K13/IBT/11)** in partial fulfilment of the requirement for the award of the degree of Master of Technology, Delhi Technological University (Formerly Delhi College of Engineering, University of Delhi), is an authentic record of the candidate's own work carried out by her under my guidance.

The information and data enclosed in this dissertation is original and has not been submitted elsewhere for honouring of any other degree.

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I hereby declare that this dissertation entitled **"Treatment of industrial dye waste water and its effect on growth of *Pisum sativum*"** is the record of original work carried by me under the guidance of **Dr. Navneeta Bharadvaja** and **Dr. Anil Kumar Haritash**, Assistant Professor, Department of Environmental Engineering, Delhi Technological University and this work has not been done elsewhere for the award of any other Degree/Diploma/Associate ship/fellowship/titles in this or any other University or Institution elsewhere.

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LIST OF ABBREVIATIONS

1. bw- Body weight
2. dw- Dry weight
3. H_2O_2 – Hydrogen peroxide
4. TiO_2 –Tin oxide
5. Fe^{2+} - Ferric ion
6. Fe^{3+} - Ferrous ion
7. RH -Organic compound
8. $^{*}OH$- hydroxyl radical
9. FO- Fenton oxidation
10. HCl- Hydrochloric acid
11. NaOH - Sodium hydroxide
12. UV - Ultra Violet
13. mmol - Milli molar
14. $HgCl_2$ – Mercuric chloride
15. AOSA -Association of Official Seed Analysis
16. GP - Germination percentage
17. TNC – Total non-Structural Carbohydrates

1. ABSTRACT

In India textile dyeing industry is one of the major industries that cause pollution in agricultural lands and water bodies. Effluent with higher concentrations of dyes affects the soil health and causes severe damage to the growth and quality of crops that are cultivated. Nowadays owing to global awareness and increasing concern towards deterioration of environmental quality has motivated the scientific community to carry out research to identify the pollutants that are potentially harmful to agricultural crops and organisms. The focus of this study is to identify the effect of the industrial dye (Acid orange 7) on an agriculturally important crop *Pisum sativum* (Garden peas). The present study focused mainly on to find the effects of this particular industrial dye on the crop by conducting experiments on the germination and growth of the plant. In our study samples of dye concentrations from 10 to 1000 mg/l is used for analyzing the germination and growth of *Pisum sativum*. Also this study focus on the degradation of dye by using a chemical method, Fenton oxidation and the treated effluent is also involved in the phytotoxicity analysis together with the dye and its effect on the growth and germination is studied. At low concentrations of the industrial dye the water does not affect the growth and germination but with increasing concentration the dye affects the growth considerably. The parameters studied in experiment are root length, shoot length, leave numbers , secondary root numbers, fresh weight, dry weight, germination percentage, protein content, Vitamin C content, Chlorophyll a and b content, total chlorophyll content, xanthophyll and carotene content and total non-structural carbohydrates content. From the results obtained it is concluded that the industrial dye at higher concentration is toxic to the plants. The industrial dye waste water treated with Fenton process shows less toxicity towards the plant. Hence industrial dye waste water at lower concentration can be used for irrigation after proper treatment through Fenton process.

Key words: Acid Orange 7, *Pisum sativum*, Pollution, Fenton process, Pytotoxicity, Germination

2. INTRODUCTION

Pollution is an unacceptable change in characteristics of air, land and water that may negatively affect life on earth (Odum, 1960). Due to unplanned rapid industrial growth environmental pollution occurs. Every industry is associated with pollution directly or indirectly. The industrial pollutants are the major factors involved in the alteration in physico-chemical and biological properties of the environment.

Industrial effluents discharged into nearby water bodies by make them undesirable and toxic due the presence of dyes, toxic chemicals, radioactive wastes, oils, greases, suspended solids and thermal pollutants. The concentrations of pollutants depend up on raw materials used and the quantity of water discharged into water bodies or land scapes.

Textile dyeing industry is one of the major water consuming and high polluting industries in India. Textile dyeing industries are the main reason for water pollution by discharge untreated or partially treated effluent into water bodies situated nearby.

A variety of new synthetic dyes are developed and utilized in modern industries they are acid dye, disperse dyes, mordant dyes, cationic dyes, sulphur dyes and reactive dyes (Hemalatha et al., 1997). These dyes are utilized in dyeing industries in large volumes and very amount is used to color foods, drugs and cosmetics. During the dyeing process, water is used in large quantities and they colored waste water is discarded as effluent. The aesthetic acceptability of water bodies are affected when these effluents are discharged into them. Nowadays due to water scarcity the recycling of waste water for irrigational purposes mainly considered so that can solve the disposal problem as well as used as the fertilizer needed for the growing crops (Noorjahan et al., 2003). The effluents may contain organic and inorganics that can increase the growth and yield of commercial crops. Beneficial effects of effluents on the growth of crop plants have been reported in many reports. But the crops get affected if the effluent is used in higher concentrations (Sundaramoorthy and Kunjithapatham, 2000).

When the raw dyeing factory effluent at different concentrations is used for irrigation it reduced the germination of crops and also reduced the concentration of important pigments and biomolecules and the yield of pulses and cereals can be increased if the effluent is used after treatment. (Parameswari and Udayasoorian, 2013).

Waste water from dyeing industries impact the aquatic environments strongly. Dyes from the textile and dyeing industries are very difficult to treat because of its complexity in the chemical structure. Pollution due to textile industries has now become a serious treat due to the increased demands in textile products and the use of synthetic dyes is proportional to demand (Dos Santos et al., 2007). In our country water shortage is the major limiting factor in agriculture, the effluent mixed polluted water is used for irrigation of crops (Singh *et al.*, 1985). Since these polluted water is used for cultivating the crops now it becomes necessary to conduct experiments to analyze the impacts of these pollutants on the crops before it is recommended for the use in agriculture.

Seed germination is the first step in the growth of plant and that determines the establishment of plants. Muhammad and Khan reported that the percentage of seed of kidney bean and ladies fingers when grown with industrial effluent. With the experimental studies on *Cicer arietinum* Dayama (1987) observed that in Bengal gram at higher concentrations of effluent the percentange of seed germination is low but at highly diluted effluent concentrations the germination was found to increase. In *Cicer arietinum* L. when the effluent concentration is very low the growth of plant is comparatively more the control but the germination started to reduce wityh the increase in concentration (Hedge and Hofreiter, 1962).

The chemical content of the effluent of textile industry made it toxic for plants, animals and fishes. Hence these effluents should not be used in agriculture without proper treatment. (Susan Verghese and Kumar, 2004).

3. LITERATURE REVIEW

3.1. *Pisum sativum L.* - Garden pea

The garden pea today we consume is originated from central Asia and the Middle East (Press *et al.,* 2000). The green pea is an important food crop which is being cultivated from ancient times(Fernald,1950).Peas are now cultivated all over the world and almost in all climates and it is consumed in dry and fresh forms. Every year nearly 3 million tons of peas is cultivated (Miles*et al.,*2004).Currently Canada is the largest producer and exporter of peas in the world. India, France, Russia and China also produce peas in large scale. India is one of leading countries in export and import of peas because its large consumption of this vegetable. (Rohit*et al.,*2006).

3.1.1. Scientific name

Classification of green peas	
Kingdom	**Plantae**
Division	**Magnoliophyta**
Order	**Fabales**
Family	**Fabaceae**
Genus	***Pisum***
Species	***Sativum***
Botanical name	
Pisum sativum	

3.1.2. Characteristics of pea plant

P. sativum is seasonal crop which is cultivated in cold season. This crop is grown annually and it has a life span of one year. Pea plants grow usually up to 1-2 m (Voss,1985).These plants grow in a temperature range of 13 to 25 °C. They cannot withstand the heat of summer and they are not grown in the warmer areas. Harvesting of these crops is usually carries out after 60 days for

planting. This crop can only be grown at low temperature areas, the crop is damaged when grown at higher tempertures (Hernandez, 1992).

3.1.3. Plant morphology

Pisum sativum is a herb and its **leaves** are alternate and pinnately compound. The leaflets of *Pisum sativum* have dimensions of 1.5 to 6 cm length × 1to 4 cm width. The leaflets are ovate and entire. It has four pairs of leaves on each side. The stems is round and slender which extends in the length up to 10 cm (Voss,1985).

Figure 1: ***Pisum sativum***

Flowers is uaually 1.5-3.5cm long. Calyx looks like a tube it has length of 8-15mm. Corollas has white or pink, or purple colour (Voss,1985). The flowers have 5 sepals and 5 petals with 10 stamens and one carpel. The petal is 1.6 -3cm long. The style is curved, grooved longitudinally and flattened. The plant usually flowers from may to September. The plants pollinate through insects like bees (Fernald,1950) and also by self-pollination.

The plant produce leguminous fruit and it is usually 4-15 cm long and 1.5- 2.5 cm wide. The fruits have pods and each pod contain 2-10 seeds (Voss,1985).

Seeds have colors like white, green, brown or grey. Like most of angiosperms the mature seeds are without endosperm.For germinating seeds the cotyledons serve as nutrition (Leubner, 2007).

3.1.4. Importance of *Pisum sativum*

- It has edible pods and it is a common vegetable in many countries (Voss,1985).
- The oil extracted from the seeds of the plant is used as contraceptive.
- The seeds of this plant in powdered form is used to treat skin allergy (Fernald,1950).
- The flowers of these plants are also edible (Badertscher and Newman 2003).
- The tendrils of pea plants are used for decoration of food stuffs (Travis,1999).
- Green peas are also an environmental friendly food. Pea crops fix atmospheric nitrogen gas to soil as nitrates and nitrites that can be used by the plants as a source of nitrogen and hence it reduces the use of nitrogen fertilizers. Also the root system of the plant is very shallow and thus it increase the soil porosity also prevents the erosion of soil. Pest problem is low in pea production. These are the benefits of pea cultivation (Duke,1981).

3.2. Industrial dyes

Colored substance which has affinity for the substrate that come in contact with them is called dye. Industrial dye is a substance that adds color to textiles,fabrics, paper, plastics, etc. They impart color by mechanism like dispersion, absorption and chemical reactions. Each Dye has different characteristics like affinity for different substrates, resistance to sunlight, washing, perspiration etc., (Booth and Gerald ,2000). Dyes are prepared and applied from ancient times. Dyes are produced from natural stuffs like plants(roots, berries, flower or leaves),trees (barks),lichens, insects, mollusks, guano (bird excreta) and minerals. William Henry Perkin discovered synthetic dye in 1856 called mauveine (Garfield,2000). After that thousands of synthetic dyes are prepared.Due to its less cost, range of colors,better imparting nature synthetic dyes started to replace the natural dyes in a faster rate.

3.2.1. Classification of industrial dyes

Majority of the dyes that are produced are utilized by the textile and dyeing industry. Based on their performance the dyes are classified in industries (Hunger, 2003). Most of the dyes used today in industries are azo dyes.

The industrial dyes are classified as

- •Protein Textile Dyes
- •Cellulose Textile Dyes
- • Synthetic Textile Dyes

3.2.1.1. Cellulose Textile Dyes

Direct dyes

As the name indicates these dyes impart color to the fabrics without any fixation. They are mostly azo dyes, and little similar to acid dyes. Sulphate group of this dye increases the solubility of this dye and it also helps in tight binding with the fabrics through bonds like hydrogen bonds , dipole –dipole interaction and Vanderwaals force. Direct dyes reduce the use of mordant to color cellulose fibers. Rayon, nylon, silk, cotton and wool can also be dyed using direct dyes. These dyes have poor fastness to washing and they are cheap.These dyes not very bright.

Vat dyes

Vat dyes are hybrid between dyes and pigments. They have ring structures that help to increase the interaction between dye and fiber. Vat dyes are not soluble in water. Theycannot dye fibers directly. But in alkaline solution it gets reduced and gets affixed to the textile fibers. The dye returns to its insoluble form upon exposure to oxidation or exposure to air. Indigo is an example of vat dye. Materials like nylon, linen, polyesters, wool, cotton, acrylics and rayon can be dyed using vat dyes. They are used together with mordant.

Basic dyes

Basic dyes are used to dye wool in an alkaline bath and they possess cationic functional groups. Basic dyes perform well on acrylics but poor for natural fibers. Basic dyes are water-soluble and are often used with a mordant. The dyes are set on fabrics by the formation of insoluble compounds with the help of a helper chemical called mordant. Fabrics like cotton, linen, acetate, nylon, polyesters, acrylics and mod acrylics are dyed with basic dye together with mordant. The fabrics dyed with acid dyes are then dyed with basic dyes to get better properties and appearance.

Fiber-Reactive Dyes

A fiber-reactive dye form covalent bond with fibers and it is very difficult to remove them once attached. Initially reactive dyes are produced for cellulose fibers but nowadays fiber-reactive dyes for protein and polyamide fibers are also available. They are applied in alkaline or neutral environments. Application of heat is used for shades development. Washing with soap is used to remove the unfixed dye after the treatment is complete.

3.2.1.2. Protein Textile Dyes

Acid dyes

Acidic dyes are soluble in water at faster rate. Fastness to light of acid dyes is great. The reactions involved in dyeing is ionic interaction. Acid dyes are applied for wool, animal fibers, and some manufactured fibers.

Mordant dyes

These dyes are acidic in behavior. Sodium or potassium di chromates are used together with these dyes. They are mainly used for wool and rarely used for cotton, linen, silk, rayon and nylon.

3.2.1.3. Synthetic Textile Dyes

Disperse dyes

Solubility of these dyes is low in water, they form dispersed particles to interact with fabrics. Polyesters are mainly dyed using these dyes. These dye are usually applied under high pressure and temperature. Dipole interaction and Vander waal forces are involved in the process of dyeing. Finely ground powder of these dyes is used for coloring purpose. These dyes are volatile in nature.

Sulfur Dyes

These dyes are not soluble in normal condition and in the presence of caustic soda it can be solubilized. The process of dyeing is carried out at high temperature and it is oxidized in air after the treatment. Subsequent washing removes the extra dye.

3.2.2. Toxicity of Dyes

The residual dye present in the textile effluent creates many problems in the environment. They affect the photosynthesis of alage and other aquatic plants by blocking sun light and prevents oxygen uptake of water (Zaharia et al., 2009,2011). Accumulation of toxic compound in the fishes can occur due heavy pollution of water bodies. Due to their complex structures the most of dyes are not degraded and possess carcinogenic action and hence it causes allergies, skin irritation and dermatitis. Most of the azo dyes are aromatic in nature and they have both acute and chronic toxicity. Plenty of health risk is associated with azo dyes ,they can cause problems in lungs, skin, intestine and can also prevent the formation of blood cells. Azo dyes can damage the genetic material and cause tumors. Even the best known azo dyes n induce cancer in humans and animals much toxicity analysis reveals even less than 1mg/l concentration of dye can affect fishes (Cooper, 1995).

Carcinogenicity

The dyes are toxic and they have potential to cause cancer (Novotny *et. al*., 2006, and Mathur and Bhatnagar, 2007). This is due to the presence of toxic chemicals that can damage the genetic material (Vogel, 1982). The azo dyes are reduced to produces amines which are very toxic and are carcinogens (Chen, 2006). It is widely known that azo dyes are potential carcinogens carcinogenicity (Weisberger, 2002, Umbuzeiro et. al., 2005). It is known fron many scientific researches that azo dyes are linked with bladder (Mendevedev, 1988 and Percy et. al., 1989). Malachite green produces significant effects on immune system and they possess potential genotoxic effect (Srivastav *et. al.,* 2004). Disperse blue dye can cause frame-shift mutation in Salmonella (Umbuzeiro et. al., 2005). The genotoxic and cytotoxic effect of this dye on human cells have extensively researched (Tsuboy et. al., 2007).

3.3. Acid Orange 7

Acid dyes are easily soluble in water. It can attach to fibers by ionic interaction. They can be used to dye natural as wells as synthetic fibers. It finds it application in industries like plastic, papers, leathers ad inks. They produce orange to pink color.They are used for staining cells and also used in food and drug industries for making colors.

3.3.1. Properties

Name

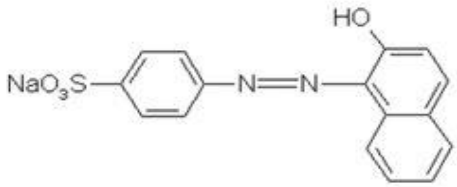
- Acid orange 7

Commercial names

- Orange 205
- Acid Orange 7 monosodium salt
- Orange II
- D&C Orange 4
- CI 15510
- COLIPA C015

Structural formula

- Monoazo class



Empirical formula

- $C_{16}H_{11}N_2NaSO_4$

Physical form

- Orange powder
- Odourless

Molecular weight

- 350.3 g/mol

Manufacturing Methods

- 4-Aminobenzenesulfonic acid diazoand Naphthalen-2-ol coupling

Orange acid dyes examples

- Ethyl orange Sodium salt (CAS RN: 62758-12-7)
- Orange II (CAS RN: 633-96-5)
- Orange III (methyl orange, CAS RN: 547-58-0)
- Orange IV (CAS RN: 554-73-4)0
- Orange G (CAS RN: 1936-15-8)
- Tropaeolin O (CAS RN: 547-57-9)
- Victoria orange
- Acid Orange II (Dibromofluorescein, 596-03-2)

3.3.2. Toxicity analysis of Acid Orange 7

Acid orange 7 shows **acute or oral toxicity** towards mice, rat and dog. In the studies made on rats and mice lethal dosage 50 is observed to be > 10,000 mg/kg bw(body weight)(Dipaliand Saha,1981, Singh and Khanna,1987) . Similarly on the research with dogs the lethal dosage 50 is observed to be > 1000 mg/kg bw (Report 81998, 1961). From these studies it is concluded that the dye is toxic at higher concentrations but with lower concentrations it has no observable effects.

Studies conducted on 4 male albino rabbits with 100mg, After 24 hours of application light redness in skin in few rabbits were observed due to Orange II. The substance is considered as a mild **skin irritant** (Draize*et al.*,1944 ,Dipaliand Saha,1981).

Acid Orange 7 was assayed for **gene mutations** at the *tk* locus of mouse lymphoma cells. The test was conducted on 7-8 weeks old male Syrian hamsters. At low concentrations the dye showed no observable effect and but in higher concentrations no cell survived the condition (Wollny,1999). This studies show that the dye is can cause gene mutation at higher concentration.

Skin painting **carcinogenicity** test is conducted with Acid Orange 7. 50 male and 50 female mice were taken as treatment set and 100 male and 100 female mice were taken as control. With one

percent concentration mice were painted weekly once on oral exposure in a particular area. For 18 months survival, body weight, and growth were observed. Initially fifty percent of the treated animals were taken for and then it is carried out for all tumor cells. The experiment does not show carcinogenicity at low concentrations (Steven 1984).

For analyzing the **maternal and fetal toxicity** 20 mated female rats are treated with dosages of 0, 100, 300, 1000 mg/kg bw/day. Two females died after four or five administrations of dye in1000 mg/kg bw/day group. They exhibited very poor conditions before death. In test groups the rats exhibited poor food intake and body weight reduction, changes in urine and feacal colours. In the all tested groups, enlargement of spleen occurred and the increase in spleen weight is proportional to dosage given. There is no abnormality were observed in fetus. In the mean body weight of fetus in test groups was observed to decrease (Beckerand Biedermann, 1999). Thus these experimental studies show Acid orange 7 show Maternal and fetal toxicity.

3.4. Treatment methods for industrial dyes

Tonnes of commercial dyes are produced and utilized in today's world every year (Pearce *et al.*, 2003 and McMullan *et al.,* 2001). Industrial dye Waste water contains recalcitrant organic molecules which are highly stable to light and aerobic digestion, hence they are difficult to treat. Efficient decolorization of synthetic dyes is not possible by traditional methods due to high cost involvement and disposal constraints in industries like dying, paper and textile industries (Ghoreishi and Haghighi, 2003).

3.4.1. Biological methods

Biological treatment is the cheapest method for effluent treatment. Microorganisms like yeast, algae, bacteria and fungi are used for the treatment of industrial effluents. (McMullan et al., 2001 and Fu and Viraraghavan, 2001). Biological treatment are very sensitive to variations in toxicity of chemicals and it requires a large land area and they often less flexible in design and operation (Bhattacharyya and Sharma,2003). Traditional biological treatment do not provide considerable color elimination (Robinson et al., 2001). These process degrade many organic molecules but some recalcitrant molecules remain untreated such as xenobiotic azo dyes are not completely degraded (Barr and Aust, 1994, Banet *et. al.,* 1997). Biological degradation using micro orgainsms are given specific attention (van der Zee and Villaverde, 2005). Biological treatment

utilizes aerobic and anaerobic degradation by microorganisms. Anaerobic treatment involves azoreductase enzyme sysyems (Frizters et. al., 2006). The treatment of dye wastewaters can be carried out by the combination aerobic and anaerobic bioreactors (Zee, 2005). Application of bacterial for degradation of dye (Santos et. al., 2007). Algal application (Aksu, 2005) and fungal application are extensively studied (Kaushik and Malik, 2009; Brar *et. al.*, 2006). Fungus has many advantages and hence it is widely used for treatment of effluents, mycelia of fungi provide great surface area and better contact and also fungi exhibit high level of tolerance towards effluents (Olieveira et. al., 2007). .

3.4.2. Chemical methods

Chemical methods for treatment of industrial dye waste water include coagulation or flocculation, precipitation combined flocculation, electro kinetic coagulation, electro floatation, ozone oxidation and electrochemical processes. Chemical methods are very costly and disposal of sludge is a very big problem associated with it. Because of chemical use it may lead to secondary pollution problems. Nowadays advanced treatment techniques like advanced oxidation processes which makes use of hydroxyl radicals for degrading the pollutants. Eventhough chemical methods are effective for effluent treatment, it is not commercially utilized because of its high cost . Involvement of electricity and costly chemicals are also problematic.

Chemical methods mainly are of 4 types

- Sodium hypochlorite oxidation (Lodha & Chaudhury, 2007)
- Ozone oxidation(Gahr et. al., 1994),
- Fenton's oxidation (Slokar & Marechal, 1997)
- Photochemical or electrochemical degradation (Awad & Galwa, 2005).

Commonly used dye treatment method is ozonation (Tanja et. al., 2003). During ozonation double bonds in chromophore are cleaved and thus results in decolorization. The compounds produced in ozonation are very toxic in nature(Wang et. al., 2002). Though long term ozonation remove the toxic products that are produced. Photocatalytic oxidation together with electro chlorination reduces the toxicity considerably(Wang et. al., 2008).

3.4.3. Physical methods

There are many physical methods used for treating effluents first one is adsorption, this can done using many natural stuffs (Nigam *et. al.*, 2000) and the second method is coagulation. Some costly methods include membrane filteration and ion exchange (Golob *et. al*., 2005). Some research papers also state reverse osmosis (Bastaki, 2004). Traditionally alum and are used for coagulation but nowadays inorganic polymer such as flocculants are also used (Tang, 1990). For the treatment of textile effluents nowadays chemical coagulation methods are widely employed (Yuan et. al., 2006, Zhu et. al., 2007). Various natural materials like corncobs, rice husk, pinewood, sugarcane bagasse, chitosan etc. are used for biosorption which can absorb and accumulate dyes (Crini, 2006, Ferrero, 2007). Excess sludge production is a main problem of physical methods. Dabrowski, 2001 states that adsorption is the effective method for treating industrial waste water. Adsorption and ion exchange are the two important mechanism involved in decolorisation (Slokar and Le Marechal, 1998), and particle size, surface area of adsorbent, temperature, time of contact and pH are factors play an important role in adsorption process (Kumar *et al.*, 1998). Membrane filtration process is also an effective physical method. These processes suffer from problems like membrane fouling and limited life time of membranes and hence replacement of membranes has to be done periodically.

3.5. Fenton Oxidation (FO)

Fenton Oxidation is carried out at pH less than 3.5. Production of hydroxyl radicals is enhanced in this environment. In acidic environment, with the presence of excess ferrous ions Hydrogen peroxide reacts with ferrous ions in acidic environment to produce the hydroxyl radical, $^{*}OH$. The radical thus produced attack the organic compounds and decomposes them chemically. Suspended molecules are also coagulated by this process.

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$$
$$RH + {}^{\bullet}OH \rightarrow R^{\bullet} + H_2O$$
$$R^{\bullet} + Fe^{3+} \rightarrow R^{+} + Fe^{2+}$$
$$Fe^{2+} + {}^{\bullet}OH \rightarrow Fe^{3+} + OH^-$$

Figure 2: Steps involved in Fenton oxidation

At pH higher than 4, ferric hydro compounds are produced by formation of ferric ion from ferrous ions. In basic environment decomposition of H2O2 occurs because of less stability (Kuo, 1992). These are the main reasons for maintaining low pH during Fenton oxidation. Sludge formation in this process is very less due to simultaneous coagulation and oxidation reactions. But other chemical coagulation process used for traditional effluent treatment produces more sludge. Important parameters that affects the FO are

- Ratio of $FeSO_4$ and H_2O_2
- pH
- Temperature

In FO the oxidation increases the concentration of total dissolved solids, this parameter limits the reuse, so the additional secondary process are employed to aid its removal (Ince and Tezcanli, 1999). By combination of Fenton process and coagulation the textile waste water can be treated and utilized for other application (Lin and Chen, 1997). In this case the TDS and conductivity is also removed considerably with chemical oxygen demand.

4. METHODOLOGY

The work on phytotoxicity analysis has been carried out in the Environmental Microbiology Laboratory of the Department of Environmental Engineering, Delhi Technological University. The details of experimental material and methodology followed are represented below.

4.1. Fenton process for treatment of industrial dye waste water

250 ml of Acid Orange 7 dye is taken in the conical flask in 50mg/l concentration. The pH of the dye is adjusted to 3.1 using 0.1 N HCl/0.1N NaOH. After pH adjustment Ferrous sulfate (10mg) is added to the dye. Now the reaction is initiated with 5 mmol of Hydrogen peroxide. The conical flask is kept on a mechanical stirrer and the stirrer speed is set 100 rpm. After 15 minutes the flask is removed from the stirrer. The treated water is then used for toxicity analysis.

4.2. Seed Germination Studies

4.2.1. Preparation of seeds for plating

The seeds of *Pisum sativum* were soaked in solution containing detergents like teepol or tween-20, fungicide bavistin (0.1%) and 0.2% streptomycin. After this the seeds were surface sterilized with mercuric chloride of concentration 0.1% for 5minutes and finally these seeds were washed with distilled water for 3-4times before plating.

4.2.2. Preparation of Glass wares

Glass wares were soaked in chromic acid (5.0%) solution for six hours,and then they are washed in running water. Then they are soaked in 0.1% Teepol overnight and were thoroughly washed in tap water and distilled water in steps. The glass wares were at 121^0C at 15 lbs/square inch pressure for 20 minutes.

4.2.3. Experimental design for germination studies

4.2.3.1. Plating

Plating was carried out under aseptic conditions inside laminar air flow chamber, which was first wipped with 70% ethanol, and sterilized with UV light for 20 minutes. The forceps and glassware used at the time of inoculation were first autoclaved at 120°C for 20 minutes and used.

Four seeds of pea were placed per sterilized glass petri plate lined with a filter paper disc. The filter papers were soaked with 5 ml of distilled water in case of control and 5 ml of various concentrations of dye (10 mg/l, 20 mg/l, 30 mg/l, 40 mg/l, 50 mg/l, 60 mg/l, 70 mg/l, 80 mg/l, 90 mg/l, 100 mg/l, 150 mg/l, 200 mg/l, 250mg/l, 300 mg/l, 350 mg/l, 400 mg/l, 450 mg/l and 500 mg/l and 1000mg/l) and treated dye waste water (Fenton Process).The preparation was moistened with 2 ml of dye daily and observed for radicle and plumule emergence. The experiment was done in duplicates, the results were averaged. Germination percentage, root length, shoot length, dry and fresh weight were determined. Germination percentage was calculated as described in the Association of Official Seed Analysis (AOSA, 1983)

Germination percentage (GP) = seeds germinated/total seeds inoculated x 100 .

4.2.3.2. Culture condition

The cultures were incubated in an air conditioned incubator at a temperature of 25°C under 16:8 hour light:dark photoperiods. The light was provided with cool white fluorescent tubes with irradiance of 220µE/m^2/s.

4.3. Phytotoxicity analysis

Pisum sativum seeds were germinated in pots (2 seeds/ pot) and are irrigated in the experimental design given below. The experiment was conducted in duplicates. All pots were kept in an air conditioned incubator at a temperature of 25°C under 16:8 hour light:dark photoperiods. The light was provided with cool white fluorescent tubes with irradiance of 220µE/m^2/s. Every day the plants are irrigated with 10 ml of distilled water or dye or Fenton treated waste water according to experimental design and the growth was monitored and the shoot length and number of leaves were noted. After 4 weeks the plants were uprooted and the shoot length, root length, number of roots, number of leaves, fresh weight and dry weight of the plants were measured.

4.3.1. Experimental design for phytotoxicity analysis

The standards are prepared in different concentrations of Acid Orange 7 from 10 mg/l to 100 mg/l at the regular intervals of 10 mg/l and 50 mg/l to 500 mg/l with regular increments of 50

mg/l and from 500 mg/l to 1000 mg/l with constant intervals of 100 mg/l. Distilled water is used as control and industrial dye waste water treated by Fenton oxidation process is used as one set.

4.3.2. Estimation of Chlorophyll

Chlorophyll content in green plant is an important parameter that provides the valuable information about the growth characteristics of the plant. In higher plants chlorophyll a & b are the important pigments and that varies considerably with growth conditions. Chlorophyll is soluble in organic solvents and hence in our experiment acetone was used as the extraction medium.

Fresh plant material used for chlorophyll estimation was taken and weighed. It was divided into 2 equal parts and one part was dried in oven at 150^{o}C. The another part was used for estimation. The plant material was crushed using mortar and pestle with 80% acetone for extraction of pigments. The pigments were extracted in 12 ml acetone and it was centrifuged at 2500 rpm for 15 minutes. Then the supernatant was used for estimating chlorophyll content. The absorbance was noted at the wave lengths of 665 nm and 645 nm.

The chlorophyll content was calculated using the following formula

(mg) pigment per gram dry weight(dw) = $C \times V \times f / 1000 \times L \times d$

V- Volume of acetone extract in ml

f- Fresh weight of plant material in gram

L- Light path length of the cell in spectrophotometer in cm

d- Dry weight of plant material in gram

C – Chl_a or Chl_b or Total Chl

Value of Chl_a orChl_b or Total Chl is calculated using Arnon's (1949) equations are as given below.

Chl a (gl^{-1}) = $0.0127 \times A665 - 0.00269 \times A645$

Chl b (gl^{-1}) = $0.0029 \times A665 - 0.00468 \times A645$

Total Chl (gl^{-1}) = 0.0202 × A665 + 0.00802 × A645

4.3.3. Estimation of Xanthophylls& Carotenes

Carotene and Xanthophyll are plant pigments they play an important function in the metabolism of plants. Xanthophyll are yellow pigments present in leaves they protect plant from damage from excessive sun light. Carotenes are orange photosynthetic pigments they help in photosynthesis.

Fresh plant material used for estimation carotene and xanthophyll was taken and weighed. It was divided into 2 equal parts and one part was dried in oven at 150^{o}C. The another part was used for estimation. The plant material was crushed using mortar and pestle with 80% acetone for extraction of pigments. The pigments were extracted in 12 ml acetone and it was centrifuged for 15 minutes at 2500 rpm and the supernatant obtained was used for estimating carotene and xanthophyll. The absorbance was noted at the wave lengths of 665 nm, 645 nm and 470 nm.

The carotene and xanthophyll content was calculated by the formula below

mg pigment per gram dry weight (dw) = C × V × f / 1000 × L ×d

V- Volume of acetone extract in ml

f- Fresh weight of plant material in gram

L- Light path length of the cell in spectrophotometer in cm

d- Dry weight of plant material in gram

C – Carotene & xanthophyll

Value of C is determined using the relationship

C= [(1000 ×A 470) – (3.27 ×Chlorophyll a) - (1.04 ×Chlorophyll b)] / 229

4.3.4. Estimation of Vitamin C

Vitamin C is essential for growth of plants. It serves as an antioxidant and protects the plant during stress condition. It is determined using iodometric method.

0.5 g of plant material was weighed in electronic balance and it was used for estimation. The plant material was crushed in mortar and pestle with 10 ml of 2N HCl and then it was diluted to 50 ml with distilled water. To this solution 1ml of 1% starch solution was added. After this 1ml of Potassium iodide was added. Then this mixture was titrated against 0.1 N Potassium dichromate until persistent blue colour appeared.

Concentration of vitamin C was calculated using relationship 1 ml 0.1 N potassium dichromate is equivalent to 0.008806g Vitamin (Morait, 1977)

Concentration of Vitamin C (g/100g) = V×0.008806×100/ 50× w

V- Volume of Potassium dichromate used in ml

w- Weight of plant material in g

4.3.5. Estimation of protein

Proteins in plants are very important for maintaining the structure and function of all cells. In our experiment estimation of protein is performed as described by (Lowry *et.al* 1951)

The plant material used for estimation was weighed using electronic balance and the protein was extracted using 5 ml of 90% methanol in mortar and pestle. Then this was centrifuged for 20 min at 3000 rpm to remove the cell debris from the sample. For estimating protein the supernatant was used. The standard curve for protein estimation was plotted using Bovine serum albumin. To 0.3 ml of protein solution, 3 ml of Alkaline copper sulphate reagent was added and incubated at room temperature for 10 minutes. Then 0.3 ml of Folin cio-calteau reagent (1N) was added and incubated for 30 minutes at room temperature. Finally the absorbance was noted at 660 nm.

Concentration of protein was calculated using the formula

Protein concentration (g/100g of plant material) = C× 16.666 × 100 / w

C- Concentration obtained from standard plot in mg/ml

w- Weight of plant material taken in g

4.3.6. Estimation of Total non-structural carbohydrates (TNC)

Non-structural carbohydrates are those carbohydrates they can be broken down by the enzymes present in the plants. These carbohydrates are produced during photosynthesis. Hence estimation of TNC is useful for understanding the photosynthetic ability of plants.

The plant material used for estimation was weighed using electronic balance and taken. It was crushed using mortar and pestle with 0.2N sulphuric acid and refluxed for 30 minutes at 100^0C. It is allowed to cool to room temperature and it was centrifuged to remove the cell debris. It was then diluted with distilled water to 60 ml. After dilution it was neutralized using 1 N NaOH and 1 N HCl.

Blank: 10 ml distilled water + 10 ml reagent 50

Standard: 10 ml of glucose solution of concentration 1mg/ml +10 ml reagent 50

Samples: 10 ml of neutralized plant extract + 10 ml reagent 50

In boiling water bath the sample is heated for 15 minutes and cooled to room temperature in running tap water. After cooling 2 ml of Potassium iodide oxalate solution was added and 10 ml of 1 N sulphuric acid was added. It was then transferred to titration flask and titrated against hypo solution (0.2 N). The end point was noted as disappearance of dark blue to colorless.

Percentage of TNC was calculated using the formula given below

i. Determination of glucose present in sample

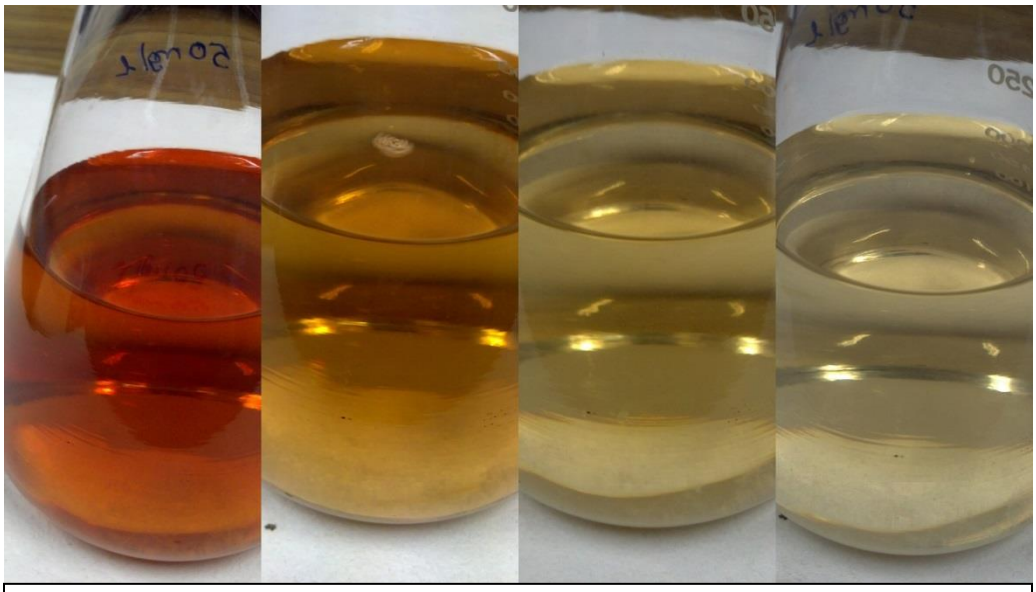
Glucose (mg) = Standard. glucose (mg)×(Blank-Sample reading)/(Blank- Standard reading)

ii. %TNC =Glucose in sample(mg)×dilution factor×100 / weight of sample(mg)

5. RESULTS & DISCUSSION

5.1. Fenton process for treatment of dye waste water

By this process the dye waste water is treated and it is decolorized up to 90 percent in 15 minutes. This treated dye waste water is utilized for estimating the effect of treated dye waste water on germination and growth of *Pisum sativum.*



a) 0 min	b) 5 min	c) 10 min	d) 15 min

Figure 3: Fenton oxidation process

5.2. Effect of dye on germination of *Pisum sativum*

From germination experiment results it is observed that the with the increasing concentrations of dye the germination percentage has been decreased slightly. At lower dye concentrations (20, 40, 50, 60, 70, 90 and 100mg/l) the germination percentage is 100. But at higher concentrations (400,500,1000mg/l) the germination percentage is reduced to 75. Although there is no specific trend in reduction pattern but the overall trend appears to be declining. In control and treated dye waste water (F) seeds show 100 % germination.

Figure 4: Seeds germinated at dye concentrations 10 -60 mg/l and control

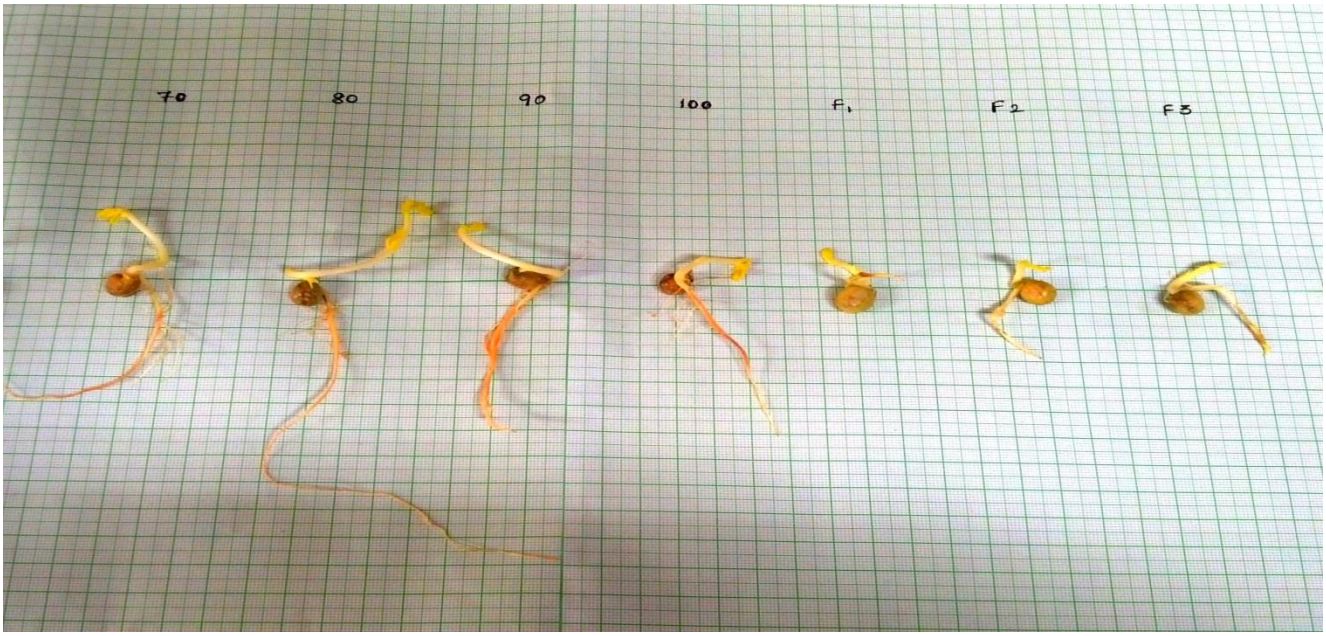


Figure 5: Seeds germinated at dye concentrations 70 -100 mg/l and Fenton treated water

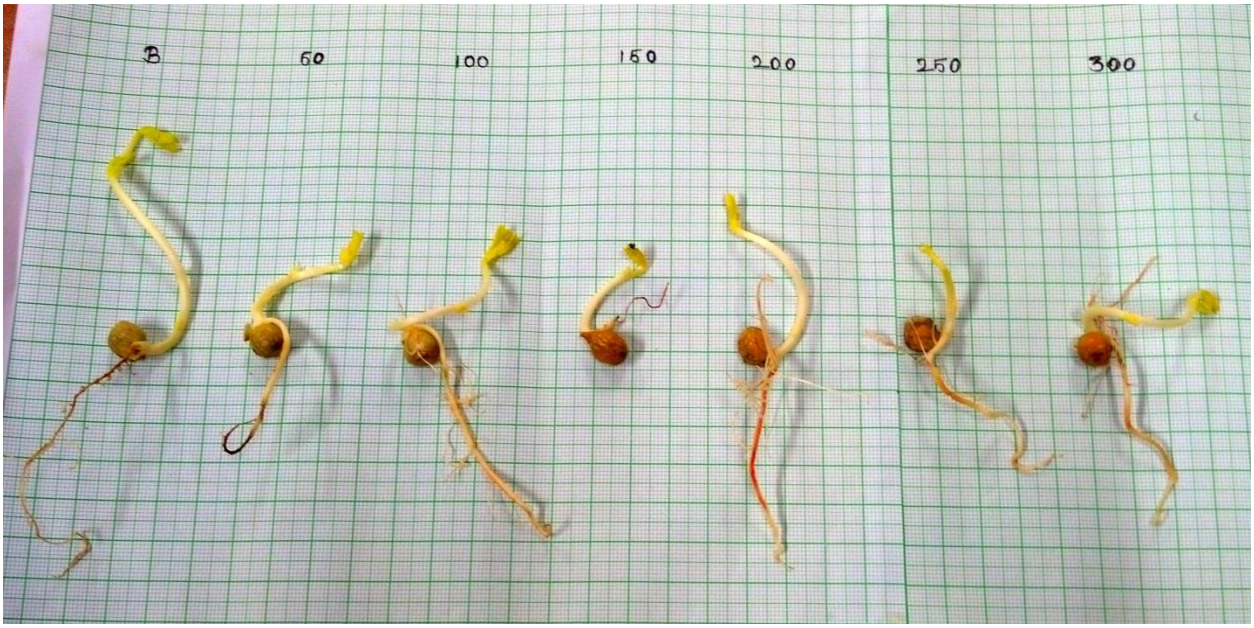


Figure 6: Seeds germinated at dye concentrations 50 -300 mg/l and control

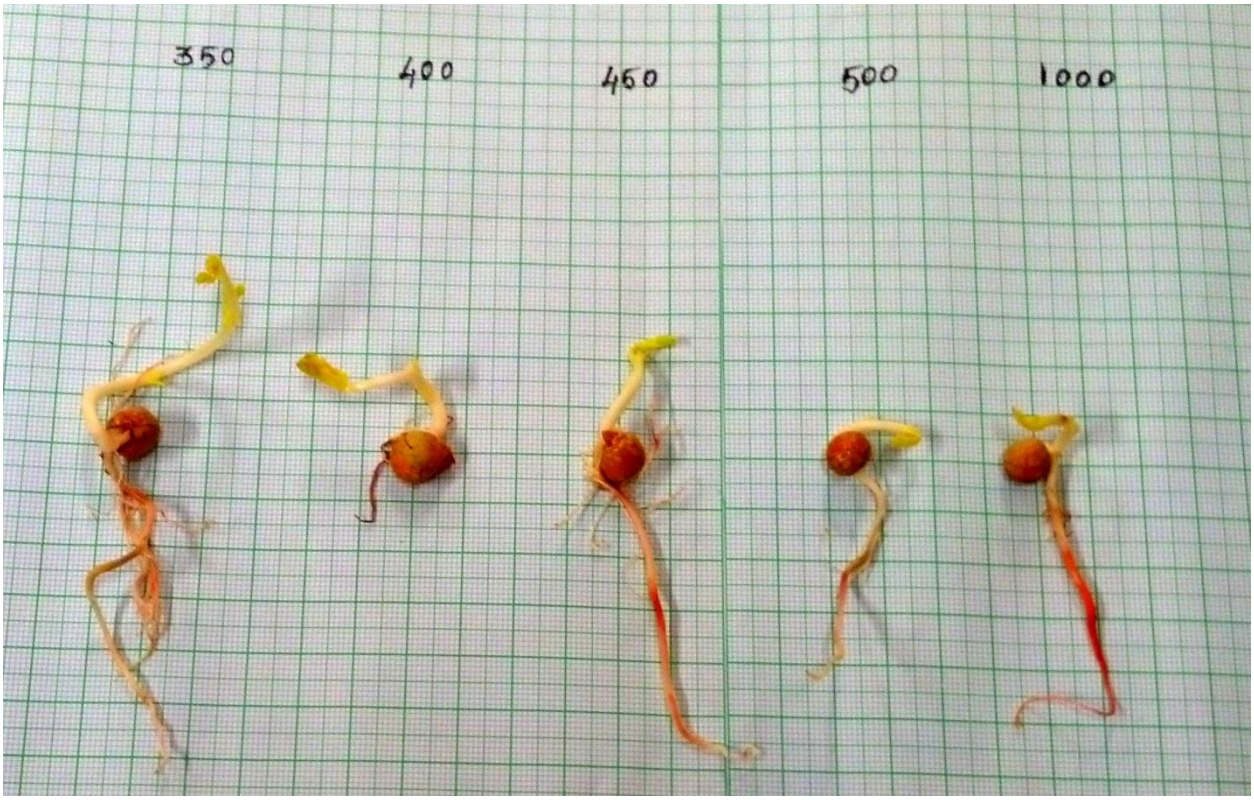
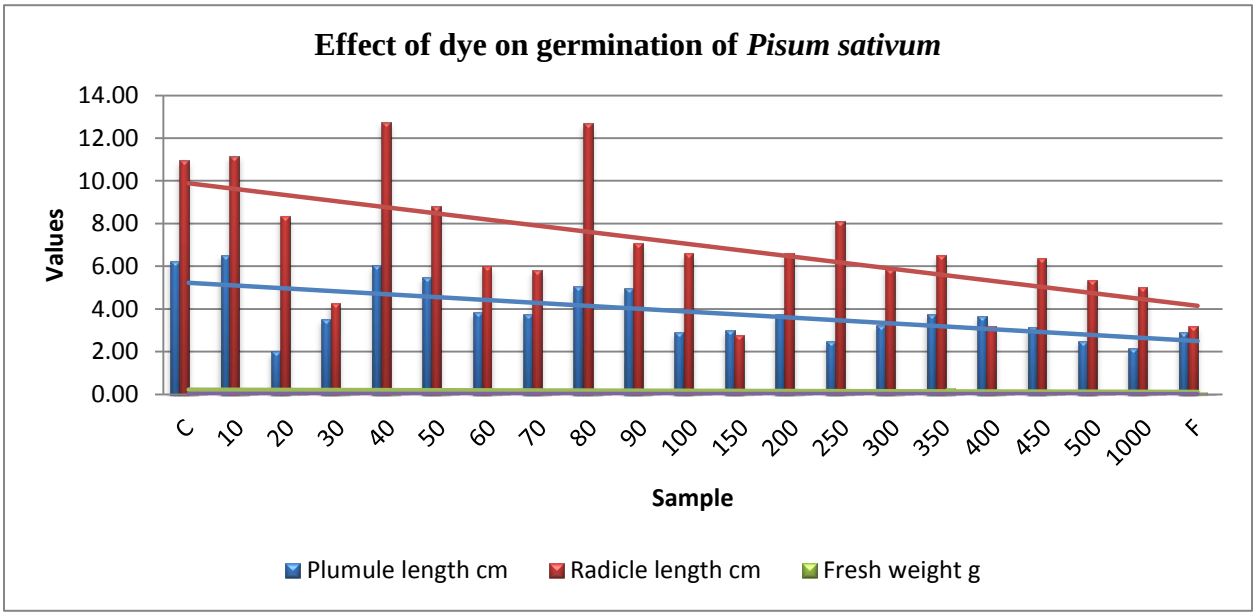


Figure 7: Seeds germinated at dye concentrations 350 - 1000 mg/l

Table 1: Effect of dye on germination of *Pisum sativum*						
Sample	Plumule length (cm)	Radicle length (cm)	No. of seeds germinated	% germination	Fresh weight (g)	dry weight (g)
C	6.25	10.95	4	100	0.27	0.02
10	6.50	11.17	3	75	0.26	0.02
20	2.03	8.35	4	100	0.06	0.00
30	3.50	4.25	2	50	0.19	0.02
40	6.05	12.75	4	100	0.22	0.02
50	5.50	8.80	4	100	0.20	0.02
60	3.85	6.00	4	100	0.13	0.01
70	3.75	5.80	4	100	0.18	0.01
80	5.07	12.70	3	75	0.27	0.02
90	4.98	7.08	4	100	0.21	0.02
100	2.90	6.60	4	100	0.10	0.01
150	3.00	2.75	2	50	0.11	0.01
200	3.75	6.63	4	100	0.20	0.02
250	2.50	8.13	4	100	0.10	0.01
300	3.25	5.88	4	100	0.21	0.02
350	3.75	6.50	4	100	0.29	0.02
400	3.67	3.17	3	75	0.19	0.02
450	3.13	6.38	4	100	0.11	0.01
500	2.50	5.33	3	75	0.07	0.01
1000	2.17	5.00	3	75	0.08	0.01
F	2.92	3.18	4	100	0.08	0.01

The fresh weight and dry weight of the germinated seeds also follows the same pattern of reduction from lower dye concentration to higher dye concentration. In control and lower dye concentrations the plant produces more biomass compared to higher dye concentrations and treated dye waste water. The overall trend line shows negative slope in fresh weight and dry weight.



Graph 1: Effect of dye on germination of *Pisum sativum*

The radicle length is longer in lower concentrations of dye and in control. The length decreases with increasing concentrations of dye. In treated dye waste water the radicle length appears short. At concentrations (10, 40 and 80 mg/l) the length of radicle is greater than that observed in control.

The plumule length observed is maximum at (control, 10 and 40 mg/l) and the radicles are longer at lower concentrations of dye than with the higher concentrations of dyes. In treated dye waste water also the radicle growth is short. The overall trend in radicle length shows decline in length from lower concentrations of dye to higher concentrations.

5.3. Effect of dye on growth of *Pisum sativum*

The shoot length of plants does not show much effect with dye treatments almost all the shoots grow above the average height. But the maximum length is observed to be 13.5 cm which is 0.5 cm more than that of control. The minimum length is observed at concentration (900 mg/l) and is 5 cm. The root length didn't follow any particular trend and the lengths are variable. The maximum root length is observed at concentration 70mg/l and the length is 14 cm.

a) Control b)10 mg/l c)50 mg/l d)100mg/l e)500 mg/l f)1000 mg/l g) Fenton

Figure 8: Plants grown at the different concentration of and Fenton treated water

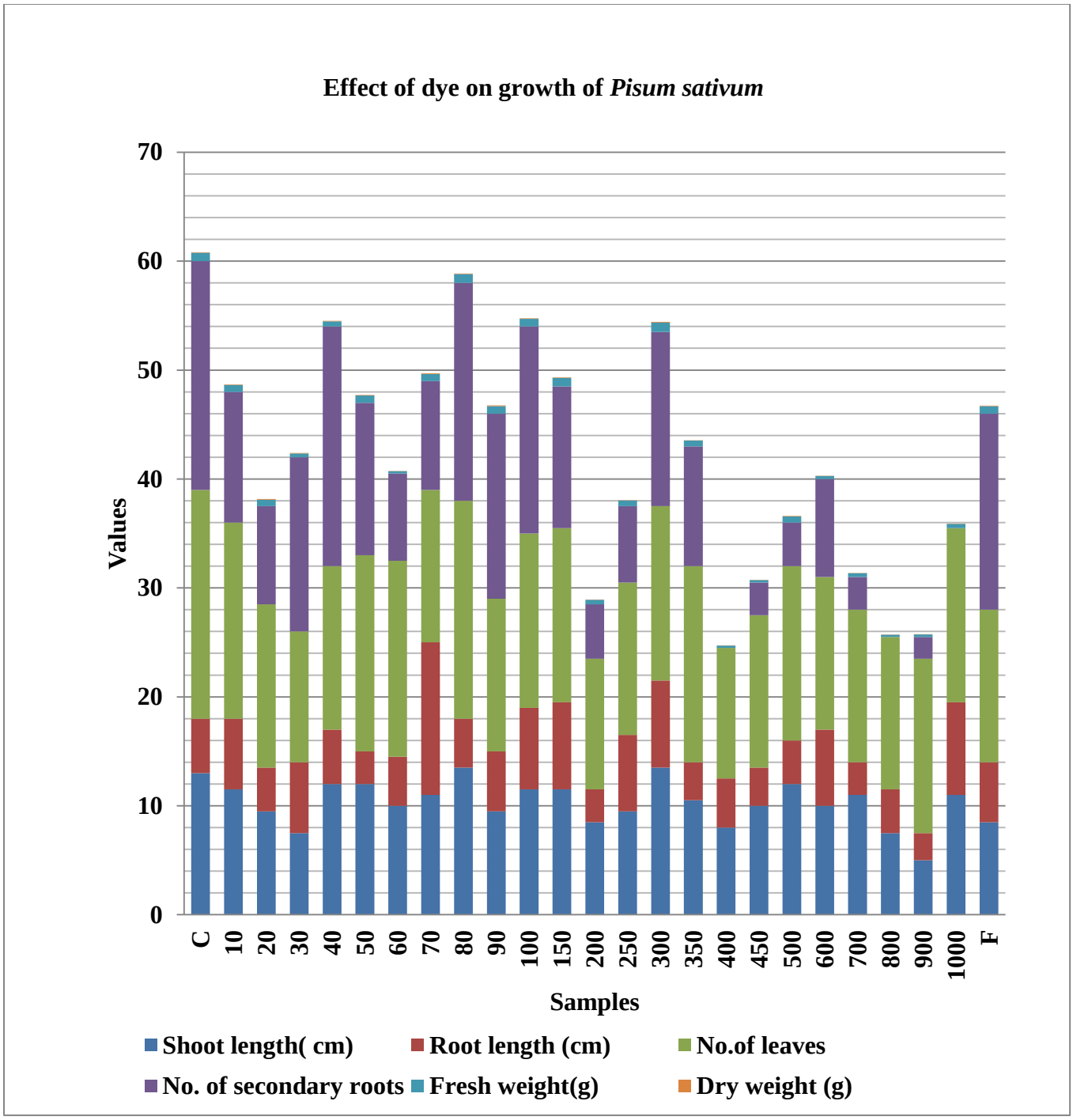
Table 2: Effect of dye on growth of *Pisum sativum*

Sample	Shoot length (cm)	Root length (cm)	No.of leaves	No. of secondary roots	Fresh weight (g)	Dry weight (g)
C	13	5	21	21	0.76	0.06
10	11.5	6.5	18	12	0.65	0.05
20	9.5	4	15	9	0.60	0.05
30	7.5	6.5	12	16	0.36	0.03
40	12	5	15	22	0.48	0.04
50	12	3	18	14	0.65	0.05
60	10	4.5	18	8	0.21	0.02
70	11	14	14	10	0.65	0.05
80	13.5	4.5	20	20	0.81	0.06
90	9.5	5.5	14	17	0.70	0.06
100	11.5	7.5	16	19	0.71	0.06
150	11.5	8	16	13	0.78	0.06

200	8.5	3	12	5	0.41	0.03
250	9.5	7	14	7	0.52	0.04
300	13.5	8	16	16	0.85	0.07
350	10.5	3.5	18	11	0.52	0.04
400	8	4.5	12	0	0.21	0.02
450	10	3.5	14	3	0.24	0.02
500	12	4	16	4	0.55	0.04
600	10	7	14	9	0.27	0.02
700	11	3	14	3	0.35	0.03
800	7.5	4	14	0	0.22	0.02
900	5	2.5	16	2	0.22	0.02
1000	11	8.5	16	0	0.37	0.03
Fenton	8.5	5.5	14	18	0.68	0.05

The biomass of the plants that are grown in lower concentrations of dye and control are comparatively more than that are grown in higher concentrations of dye. The maximum weights of plants are observed in (control, 80,150 and 300 mg/l) and the minimum weights of plants are observed in (400,800and 900 mg/l). The plants grown in treated waste water show biomass production comparable with control plants.

The secondary roots are higher in number at lower concentrations of dye and in control. But as the concentration of dye increases the number of secondary roots reduced significantly. At concentrations 400,800 and 1000 mg/l there is no secondary roots present at all. The number of secondary roots present in control is 21 and in treated dye waste water the plant produced 18 secondary roots which very near to the control. The growth secondary roots are affected significantly by the dye. The number of leaves produced in all the plants are more nearly same. The dye did not produce any effect on number of leaves. The trend in leave number is almost linear. The maximum number of leaves is produced in control (21 leaves) and the minimum number of leaves produced is 12.



Graph 2: Effect of dye on growth of *Pisum sativum*

5.4. Estimation of Chlorophyll

The photosynthetic pigments Chlorophyll a, b and total chlorophyll are estimated to analyze the effects of dye on the photosynthesis of plants. The concentration of Chlorophyll a is maximum at

250 mg/l and there is a overall decrease in concentration of Chlorophyll a at higher concentrations of dye. The trend line appears to have slight negative slope.

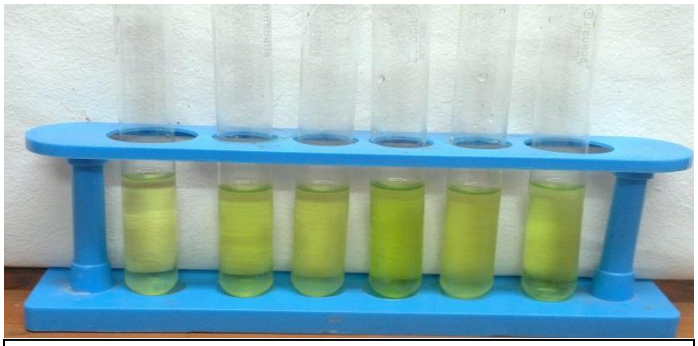


Figure 9: Samples of Chlorophyll estimation

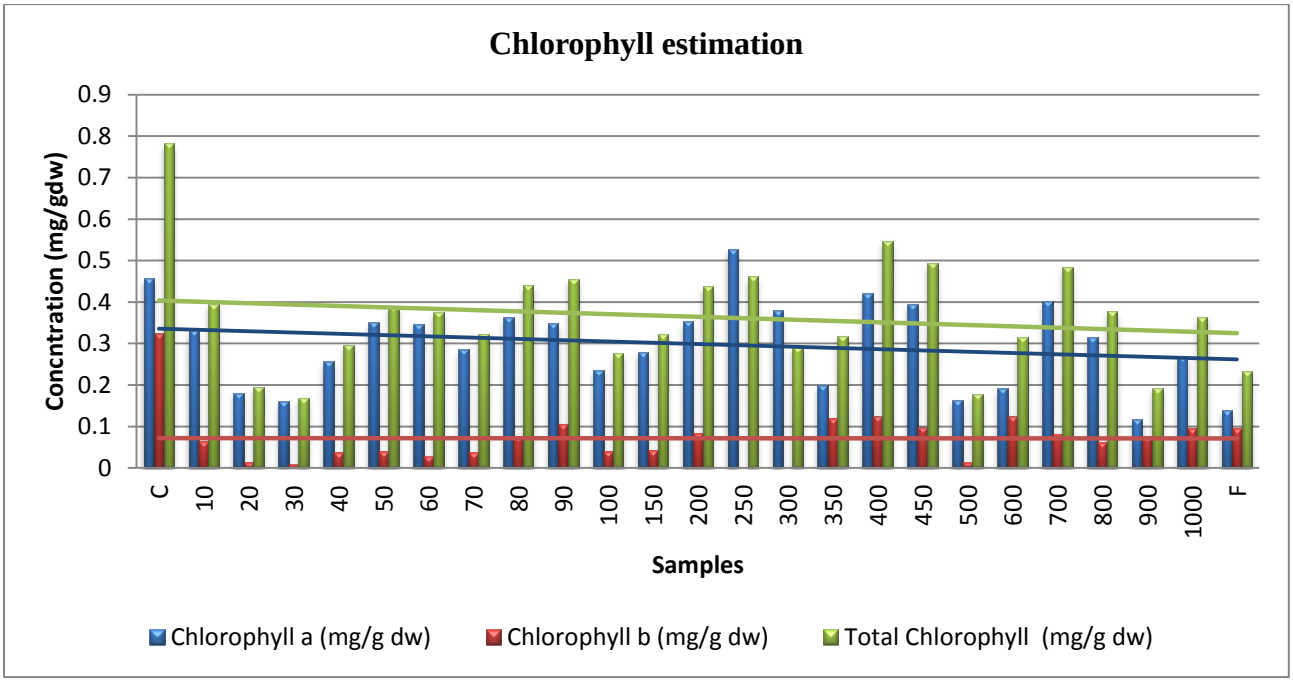
The chlorophyll b concentration is maximum at control with concentration of 0.32mg/g dw(dry weight of plant material) but the concentrations in other plants are very much minimum compared to the control. But the trend line is linear indicating the strong negative effect of dye on plant even at lower concentrations of dyes.

Table 3: CHLOROPHYLL ESTIMATION

SAMPLE	Chlorophyll a (mg/g dw)	Chlorophyll b (mg/g dw)	Total Chlorophyll (mg/g dw)
C	0.457	0.325	0.782
10	0.332	0.064	0.396
20	0.181	0.014	0.195
30	0.161	0.008	0.169
40	0.257	0.037	0.295
50	0.351	0.039	0.390
60	0.347	0.029	0.376
70	0.285	0.037	0.323
80	0.363	0.077	0.440
90	0.349	0.106	0.455
100	0.236	0.040	0.276
150	0.279	0.043	0.322
200	0.354	0.084	0.437
250	0.527	0.000	0.462
300	0.379	0.000	0.292
350	0.199	0.119	0.318
400	0.421	0.125	0.546
450	0.395	0.098	0.493

500	0.163	0.013	0.176
600	0.191	0.125	0.316
700	0.403	0.081	0.483
800	0.315	0.062	0.377
900	0.116	0.075	0.192
1000	0.268	0.096	0.364
F	0.138	0.095	0.233

The total chlorophyll concentration follows the similar trend as chlorophyll a concentration. But the total chlorophyll concentration is higher compared to chlorophyll a. The concentration of total chlorophyll is maximum at control and there is a overall decrease in concentration of total chlorophyll a at higher concentrations of dye. The trend line appears to have gradual decline in slope.

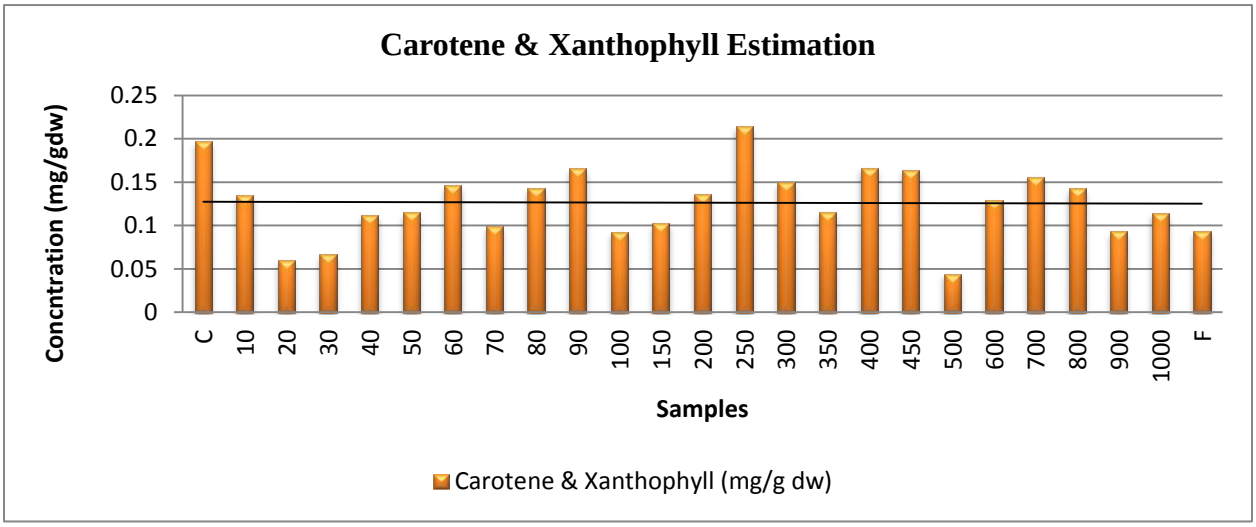


Graph 3: Estimation of Chlorophyll

5.5. Estimation of Xanthophyll & Carotenes

Xanthophylls are plant pigments that protect the plant from damage caused by excessive sunlight. They absorb wavelength that are not absorbed by chlorophyll. Carotenes are photosynthetic pigments they emit the light energy that they absorbed from chlorophyll. They

protect the plants from free radicals produced during photosynthesis. Estimation of these pigments is important to assess the health of plant and its proper growth.



Graph 4: Estimation of Carotene & Xanthophyll

Table 4: CAROTENE & XANTHOPHYLL ESTIMATION	
SAMPLE	Carotene & Xanthophyll (mg/g dw)
C	0.197
10	0.135
20	0.059
30	0.066
40	0.112
50	0.115
60	0.146
70	0.099
80	0.143
90	0.165
100	0.093
150	0.103
200	0.136
250	0.215
300	0.150
350	0.115
400	0.166
450	0.164
500	0.044

600	0.129
700	0.156
800	0.143
900	0.093
1000	0.115
F	0.093

The xanthophyll and carotene showed a slight increase in concentration at lower concentrations of dye and it reached the maximum concentration at 250 mg/l of dye and then there is a slight decrease in concentration towards higher concentrations of dye. But the overall trend line is linear. The plants grown in treated dye waste water showed minimum concentration of xanthophyll and carotene.

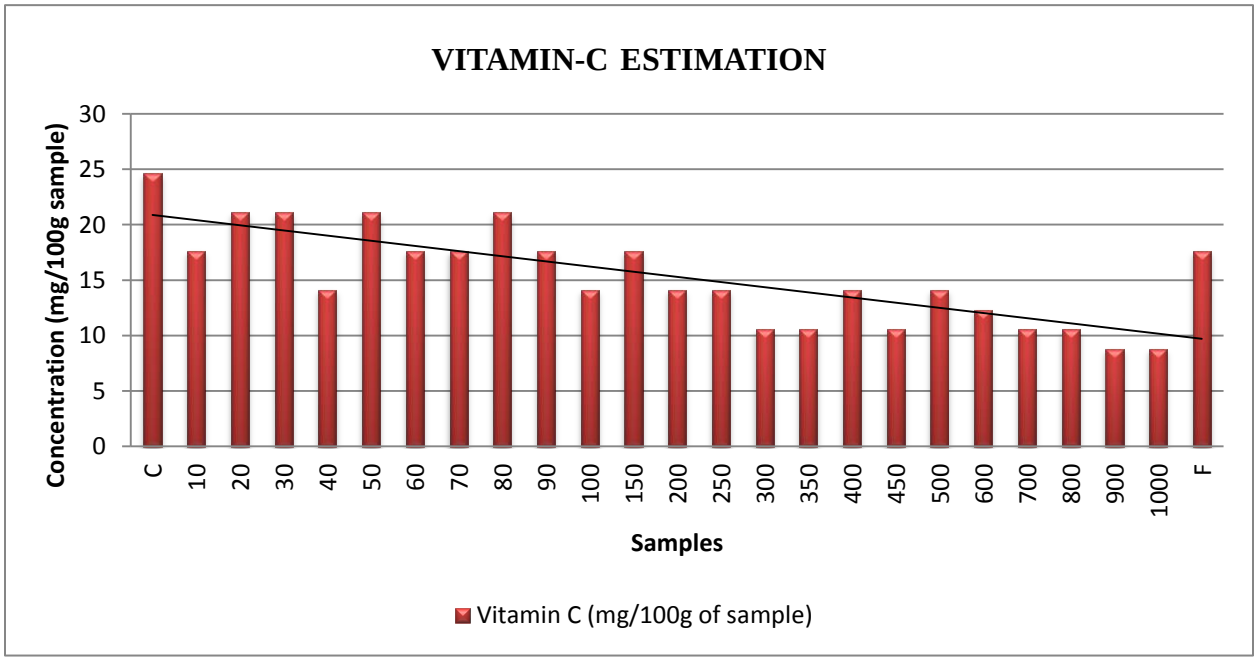
5.6. Estimation of Vitamin C

Vitamin C is an antioxidant it protects plant from free radical damage and stress. Estimation of Vitamin C gives us information about the proper growth and health of plants.

Table 5: VITAMIN-C ESTIMATION	
SAMPLE	concentration of Vitamin C (mg/100g of sample)
C	24.657
10	17.612
20	21.134
30	21.134
40	14.090
50	21.134
60	17.612
70	17.612
80	21.134
90	17.612
100	14.090
150	17.612
200	14.090
250	14.090
300	10.567
350	10.567
400	14.090
450	10.567

500	14.090
600	12.328
700	10.567
800	10.567
900	8.806
1000	8.806
F	17.612

The concentration of vitamin C is maximum in control (24.65 mg/100g of sample) and there is a decline in concentration as the concentrations of dye increases. The concentration of vitamin C is minimum at higher concentrations (900 and 1000 mg/l). This indicates that the health of plant is negatively affected at higher concentrations of dye and there is very minimum concentration of vitamin C is present to protect the plant. The trend line shows an exponential decline in concentration of vitamin C. The plant grown in treated dye waste water shows higher concentration of vitamin C (17.61mg/100g of sample). Hence it is evident that the treated dye waste water favors the positive growth and health of plants when compared with higher concentrations of dye.



Graph 5: Estimation of Vitamin C

5.7. Estimation of protein

Protein is one of the major biomolecules in the living organisms and it plays a crucial role in the plants growth and development and hence its estimation is necessary to evaluate the growth of plants.

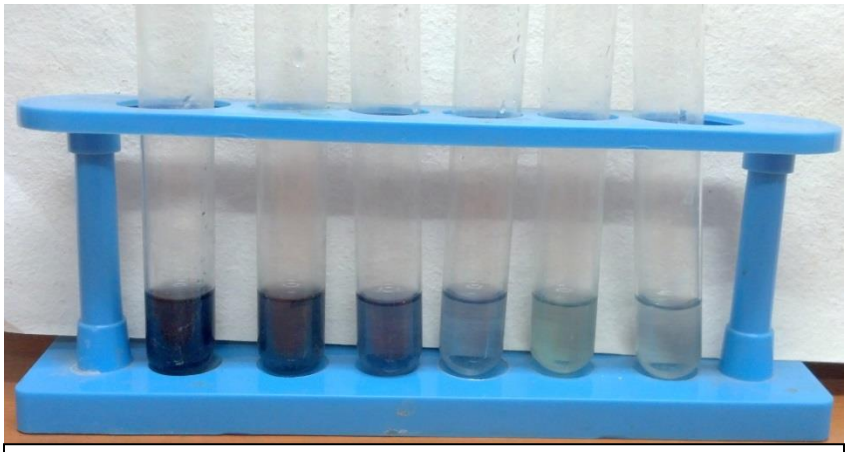
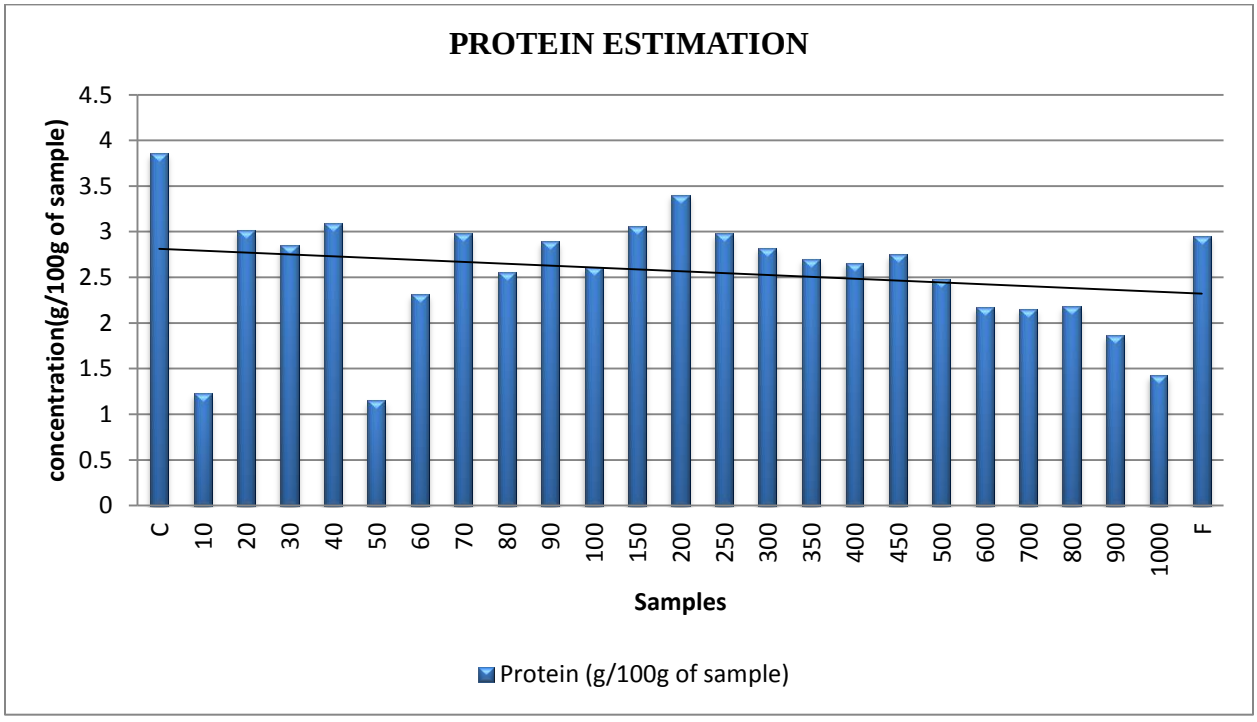


Figure 10: Samples of Protein estimation

Table 6: PROTEIN ESTIMATION	
SAMPLE	Protein (g/100g of sample)
C	3.860
10	1.230
20	3.017
30	2.849
40	3.097
50	1.153
60	2.317
70	2.987
80	2.550
90	2.897
100	2.607
150	3.057
200	3.403
250	2.980
300	2.823
350	2.703
400	2.658
450	2.747
500	2.482

600	2.168
700	2.155
800	2.182
900	1.863
1000	1.430
F	2.955

Higher concentration of protein in plants indicates the proper growth of plants. From the results it is observed that the maximum concentration of protein is present in the control plants (3.8g/100g of sample).The concentration of protein is higher in plants that are grown at lower concentrations of dye and there is a slight decrease in concentration as the concentration of dye increases. Although there is no specific pattern of decrease observed, the overall trend line shows the slight negative slope. The protein concentration in the plants grown in treated dye waste water is significantly higher (2.958g/100g of sample) when compared with higher concentrations of dye (1000mg/l) 1.42g/100g of sample.



Graph 6: Estimation of protein

5.8. Estimation of Total non-structural carbohydrates (TNC)

Carbohydrates which can be broken down by enzymes present in plants called total non-structural carbohydrates. Concentration of TNC present in the plant is directly proportional to the photosynthetic efficiency of plant. Hence estimation of TNC % indicates the proper development of plants.

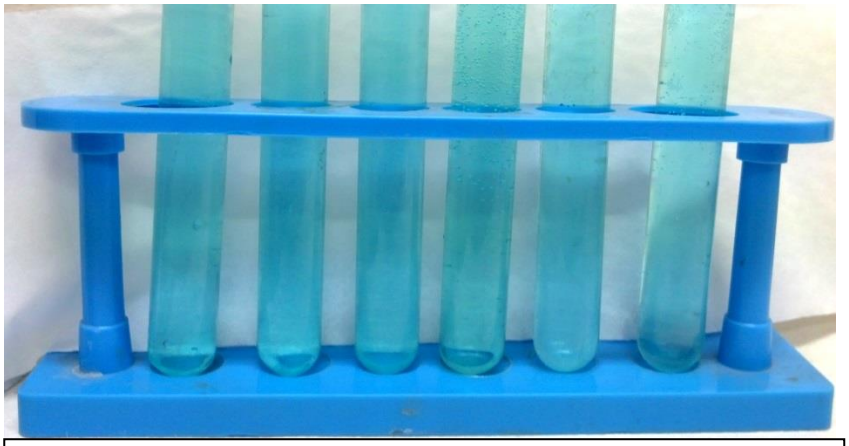
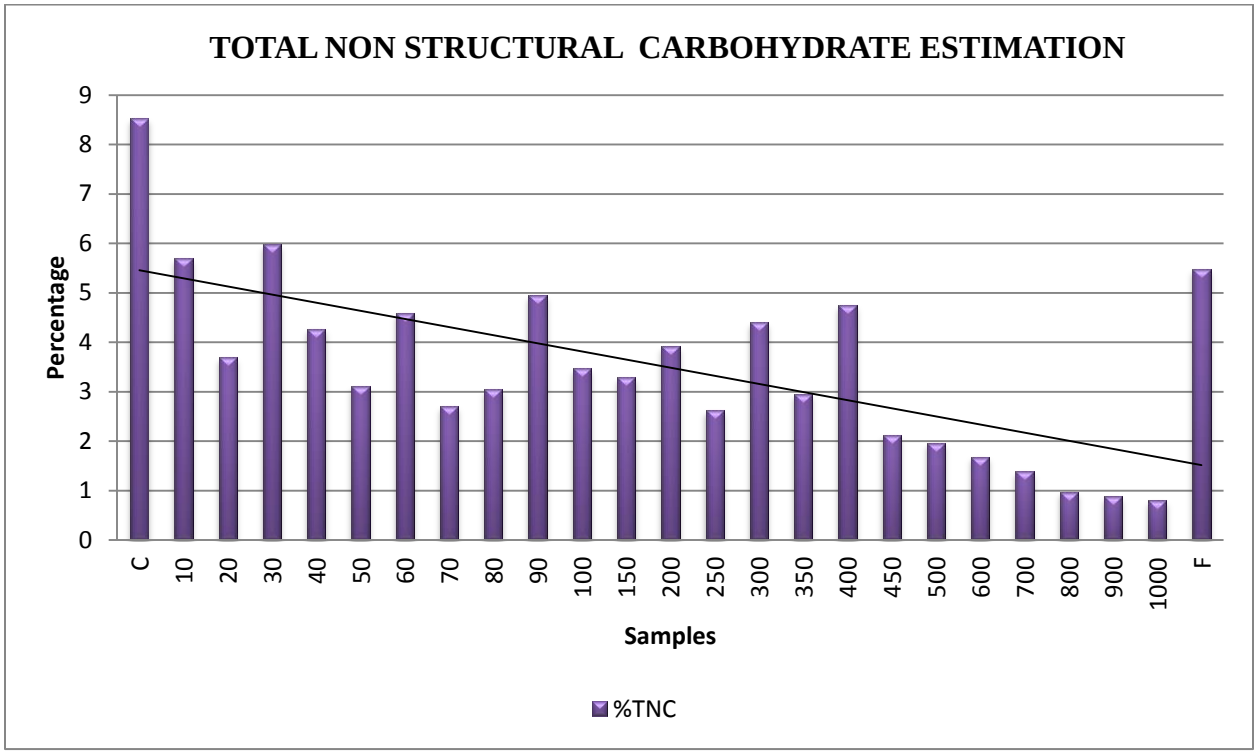


Figure 11: Samples of TNC estimation

Table 7: TOTAL NON STRUCTURAL CARBOHYDRATE ESTIMATION		
SAMPLE	Glucose (mg)	%TNC
C	1.578	8.531
10	1.578	5.692
20	1.625	3.695
30	1.099	5.968
40	1.431	4.263
50	1.834	3.105
60	1.744	4.587
70	1.990	2.694
80	1.407	3.035
90	1.483	4.941
100	1.914	3.469
150	2.132	3.285
200	1.966	3.914
250	2.184	2.619
300	1.962	4.397

350	1.132	2.948
400	0.938	4.739
450	0.800	2.119
500	0.255	1.961
600	0.398	1.680
700	0.137	1.388
800	0.180	0.976
900	0.199	0.874
1000	0.255	0.794
F	1.037	5.477



Graph 7: Estimation Total non-structural carbohydrates

The %TNC is observed maximum in control plants (8.53%) and the %TNC reduced sharply with increase in concentrations of dye. The minimum TNC % is observed in plants grown in higher concentrations of dye, in 900 mg/l TNC is observed to be 0.87% and in 1000 mg/l TNC is 0.79%. The plants grown in treated dye waste water higher percentage of TNC (5.47%) that is slightly lower than the control. The trend line of %TNC has sharp negative slope indicating the exponential decrease of %TNC with increasing concentrations of dye.

6. FUTURE PERSPECTIVE

Industrialization is linked to economic sustainability, growth and human progress. Increasing technological development increases the need for many industries. The waste from industries leads to deterioration of environmental quality and causes health hazards to plants, human and animal. Industrial pollutants cause the changes in the abiotic and biotic components of the ecosystem (Noorjahan et al., 2003).Presence of toxic chemicals in large quantities in the effluent imparts negative effects on plant development including germination and seedling growth (Baruah and Das., 1998).

In the present study the reduction in length of roots , length of shoots, dry weight and secondary root numbers and other parameters of *Pisum sativum* when grown in industrial dye waste water is observed. Similar type of tolerance studies were carried out by Sing et al., (1985) on three varieties of rice for sugar and distillery effluent and Nirmala Rani and Janardhanan, (1988) on five varieties of maize for viscose and chemical factory effluent treatment. The varietal screening of groundnut for tolerance to fertilizer factory effluent. Paper mill effluent (Sundaramoorthy and Kunjithapatham, 2000) and tannery effluent (Sundaramoorthy and Lakshmi, 2000. Simarilalrly, the screening of paddy cultivars for tolerance to tannery effluent (Lakshmi and Sundaramoorthy, 2003) and sugar mill effluent (Sundaramoorthy et al., 2003) was reported.In our experiment the chlorophyll a and b content, protein content,Vitamin C content,total non-structural carbohydrates are reduced in high concentrations of dye. Dutta and Boissys in 1999 reported that crops and vegetables are more susceptible to soil pollutants and these inhibit their growth and yield of plants. The carotene content of plant is largely affected due the dye pollution in water similar result was observed by Hemalatha and her coworkers in 1997 in which the pollutants affect the levels of protochlorophyll and carotenoid content.

The use of dyeing effluents for irrigation is beneficial alternative for reuse of water when it is used in appropriate concentration (Kumawat *et al.*,2001). The dyeing industry effluent consists of higher concentrations of many compunds which are harmful to plants(Koushik *et al.,*.2005) but these substance are beneficial for the growth of plants at lower concentration(Swaminathan and Vaidheeswaran,1991) .These reports give a positive correlations with our experimental observation that the plants in control and low concentrations of dye grown very well and the content of protein, total non–structural carbohydrates, vitamin C, Chlorophylls and xanthophylls

and carotenes are high but in high concentration of dyes these content get decreased and the growth was also minimal. Also the treated dye waste water also enhances the plant growth. Similarly many studies have been conducted on different plants to analyse the impact of dyeing industry waste water by many researchers (Dayama, 1987; Sujatha *et aL,* 1992; Himabindu and Reddy, 2005).

It is hence concluded that the textile dyeing industry effluent is toxic to crop plants. The treated and diluted effluent can be used for irrigation to grow crops without compromise considering the fact of environmental pollution and water scarcity reuse of industrial dye waste water after proper treatment is a good alternative which favors safe disposal and beneficial reuse.

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