INFLUENCE OF FERTILIZER INDUSTRY WASTEWATER ON THE GERMINATION BEHAVIOR OF BARLEY (*Hordeum vulgare*) GRAINS

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BY

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CERTIFICATE

I declare that the dissertation entitled "INFLUENCE OF FERTILIZER INDUSTRY WASTEWATER ON THE GERMINATION BEHAVIOR OF BARLEY (*Hordeum vulgare*) GRAINS" has been prepared by me under the guidance of Dr. Yogalakshmi K.N., Assistant Professor, Centre for Environmental Science and Technology, School of Environment and Earth Sciences, Central University of Punjab, Bathinda. No part of this dissertation has formed the basis for the award of any degree or fellowship previously.

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I certify that Jaskiran Kaur has prepared her dissertation entitled ” INFLUENCE OF FERTILIZER INDUSTRY WASTEWATER ON THE GERMINATION BEHAVIOR OF BARLEY (*Hordeum vulgare*) GRAINS”, for the award of M.Phil. degree of the Central University of Punjab, Bathinda under my guidance. She has carried out this work at the Centre for Environmental Science and Technology, School of Environment and Earth Sciences, Central University of Punjab, Bathinda.

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ABSTRACT

"Influence of Fertilizer industry wastewater on the germination behavior of barley (*Hordeum vulgare*) grains"

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Industries generate enormous amount of wastewater. Increased water crisis problems, coupled with stringent regulatory standards for wastewater disposal has necessitated the need for better wastewater treatment options. Most developing countries do not have sufficient funds to invest on technologies for treating wastewater. As agriculture demands more water, use of wastewater for irrigation would be the best option for treatment of wastewater. Using wastewater for irrigation purposes would solve the problem of water scarcity as well. In the present study, the effect of fertilizer industry wastewater on germination behaviour of *Hordeum vulgare* (PL-426) grains was examined. The wastewater was collected from National Fertilizer Limited, Bathinda, Punjab. The wastewater was acidic in nature and contained around 37.5% of total dissolved solids and 9.2% of total suspended solids. BOD and COD were more than that of permissible limits. The germination study was carried for a period of 6 days. For the germination study, the wastewater composition used were 0%, 25%, 50%, 75% and 100% wastewater concentrations diluted proportionately with distilled and designated as E_0-E_4, respectively. The growth of the grain was evaluated based on morphological and biochemical parameters. The effects were studied by comparing the growth at different wastewater concentration with that of control. Germination percentage, germination index, germination vigor index, root and shoot length, relative elongation ratio of shoot and root, fresh and dry weight, chlorophyll a and b, proteins and antioxidant enzymes such as peroxidase and superoxide dismutase activity of grains showed significant decrease with increase in wastewater concentration beyond 50% indicating the inhibition of germination with increase in wastewater concentration. Parameters such as dry weight, chlorophyll a and b, superoxide dismutase and proteins showed higher value at 100% wastewater than control. The results concluded that the fertilizer industry wastewater can be used effectively for the cultivation of *Hordeum vulgare* and the maximum growth could be achieved only at 50% dilution.

Jaskiran Kaur | Dr. Yogalakshmi K.N.

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Jaskiran Kaur

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LIST OF ABBREVIATIONS

Sr.No.	Full form	Abbreviation
1.	MLD	Million liter per day
2.	CPCB	Central Pollution Control Board
3.	EOE	Encyclopedia of Earth
4.	ENVIS	Environment Information System
5.	EPR	Environment Protection Rules
6.	USEPA	United States Environmental Protection Agency
7.	USGS	United States Geological Survey
8.	TS	Total Solids
9.	TDS	Total Dissolved Solids
10.	TSS	Total Suspended Solids
11.	TVS	Total Volatile Solids
12.	Ethylene Diamine Tetracetic Acid	EDTA
13.	Potassium dihydrogen phosphate	KH_2PO_4
14.	Potassium dichromate	$K_2Cr_2O_7$
15.	3, 5-Dinitrosalicylic acid	DNSA
16.	Tris (hydroxyl methyl) Amino methane	Tris
17.	Tricarboxylic Acid	TCA
18.	Standard Deviation	SD

CHAPTER 1

INTRODUCTION

Water is one of the vital necessities of life. Freshwater constitutes 2.7% of total water of which only 0.3-0.5% is available to man (USGS, 2012). However, this precious resource is continuously contaminated by our society in one or other way. Increasing population, urbanization, rapid industrialization and use of modern technology in agriculture continuously pollute the water, ultimately worsens its quality (EOE, 2012). Although industrialization has lead to economic advancement, its impact on the environment is highly pathetic and need to be addressed.

Wastewaters from industries are continuously discharged into the rivers, lakes, streams and canals which are the principal water sources for domestic and agriculture purposes. In India, at some industrial locations, untreated wastewater and hazardous wastes are injected into groundwater through infiltration ditches and injection wells (Sharma, 2005; Behera and Reddy, 2002). These resulted in change in water pH, temperature, organic, inorganic pollutants and toxic chemicals. The ecology of the environment is also badly affected by the discharged wastewater. Three main kinds of ecological risks (Ross, 1994) associated with industrial discharge are given below:

- Failure in productivity in the soil compartment
- Ground water pollution due to metal leaching
- Bioaccumulation of pollutants from wastewater through the food chain

Management of such wastewater seeks foremost awareness to avoid damage to the environment. Wastewater management is considered as more appropriate technology to deal with the ecological risk and health problems arising as a result of wastewater discharge into the nearby water bodies. A number of approaches (Kumar *et al.*, 1999) are relevant to the management of wastewater under Indian conditions. They are (1) Resource conservation such as water, energy, raw materials etc., (2) Adoption of more efficient manufacturing processes for better resource consumption, (3) To reduce the quantity and quality of wastewater that requires different treatments (primary, secondary and tertiary treatments).

Agriculture is the backbone of our country. It requires massive amount of water for irrigation. The water crisis problem has put pressure on agriculture, hampering the economic growth. Reuse of wastewater for irrigation could be a promising method to meet the demands of agriculture and water shortage, especially the need for the hour in the arid and semi arid regions because of water scarcity problem. Wastewater reuse is one of the oldest forms of water reclamation.

Reuse of treated municipal wastewater for irrigation has been successfully practiced for various crops including agronomic (Ornon and DeMalach, 1987; Tsadilas, 1997 and Burun *et al*., 2006) and horticultural (Brito *et al*., 1997; Murillo *et al*., 2000; Al-Lahham *et al*., 2003; Carter *et al*., 2005 and Lopez *et al*., 2006) crops throughout the world. Also, irrigation of forests crops using municipal wastewater has been done in many sections of the world (Al-Jamal, 2002 and Bhatti and Singh, 2003). Wastewater irrigation has several benefits in agricultural, environmental sectors and water resource management when properly planned and managed. It often results in more crop yields due to the nutrient present in wastewater.

Surface water pollution due to discharge of wastewater into water bodies can be avoided by wastewater reuse. By doing so, number of environmental problems such as eutrophication, depletion of dissolved oxygen, killing of fish and health risks like skin and neurological problems, eye irritation and blue baby syndrome can be prevented (State of the Environment Report, 2003). Though the benefits of wastewater usage for irrigation purposes are abundant, certain safety measures have to be taken in order to circumvent its short and long- term risks on the environment (Garg and Kaushik, 2008).

As per IS:2490 (part 1) -1981, the tolerance limits for discharge of industrial effluents in to inland surface waters, pH should be between 5.5-9.0, Biochemical oxygen demand should not be more than 30 mg/L, Chemical oxygen demand should be 250 mg/L, total dissolved solids and total suspended solids should not be more than 2100 mg/L and 100 mg/L, respectively.

The wastewater standards are continuously laid down because of discharge of wastewater containing huge amounts of heavy metals such as arsenic, lead, cadmium, cyanide which ultimately degrade the water quality.

Need of the Study

Increasing population, environmental degradation and decline in freshwater supply has increased the competition for water resources. In the increasing conflict between domestic and agricultural sector for water usage, it is essential to reuse the wastewater for irrigation. Everyday around 38000 MLD wastewater is generated in India (CPCB, 2009). Irrigation demands huge amount of water. Usage of wastewater for irrigation minimizes the usage of chemical fertilizers as it has its own nutrients and will be a potential source for agriculture. However, a number of environmental and health risks are associated with wastewater reuse. It is vital to examine the wastewater effect on crop growth for its efficient utilization.

Plant selection

Short germination period of 4-5 days and growth season being between 4-5 months were the main reason for selecting the barley crop. In addition, based on the literature review, to the best of our knowledge no studies have been reported on effect of fertilizer industry wastewater on barley plant.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Introduction

Water is a fundamental resource on planet. Figure 2.1 depicts the distribution of water on the earth. Only a tiny portion of earth's water (about 3%) is available to support life on earth. Of that 3%, 69% is sheltered in icecaps and glaciers, 30% is ground water and 10% as surface water in lakes and rivers (USGS, 2012).

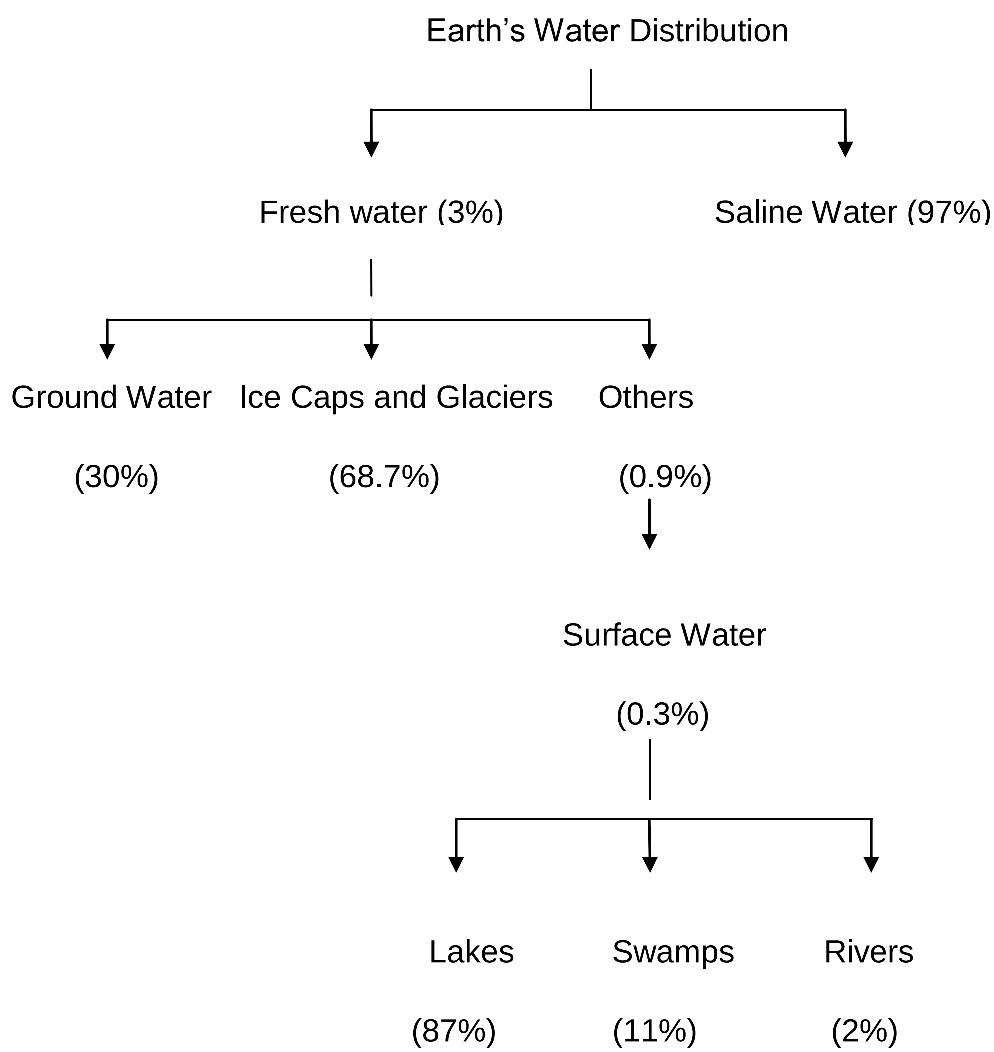

Figure 2.1 Distribution of Earth's water

Of all the freshwater taken from rivers, lakes and groundwater, 80-90% is used by Agriculture sector, the largest user of world's fresh water resources. Industries are the second major user of freshwater (around 22%) for their various process. Due to limited supply of water, agricultural sector suffers from water scarcity problem. In order to meet the challenges of increasing human population in terms of food, it becomes essential to use other forms of water for agriculture. Reutilization of wastewater for irrigation would be a promising option for meeting the problems of water shortage and wastewater disposal. However, the presence of pollutants in the wastewater and their effect on the plant growth limits its application (Shatanawi and Fayyad, 1996). The industrial effluent is complex, troublesome and rich in pollutants. They enrich the soil with toxic pollutant and heavy metals causing irreparable damage to the soil. Thus it is essential to reuse the wastewater without causing adverse impact on soil and plant growth. Several studies (Singh *et al.*, 2003; Begum *et al.*, 2010; Malaviya and Sharma, 2011) have been reported on use of wastewater for irrigation. This chapter reviews the literature pertaining to the wastewater generation by different industries and their influence on crop growth, to identify the knowledge gap especially in wastewater reuse for irrigation purpose.

2.2 Wastewater generation

A major portion of water is consumed by the industries during several processes involved in product manufacturing. The quantity of water consumed varies from industry to industry. Table 2.1 summarizes the amount of wastewater generated from different industries with their pollution load. The pollution problems were caused because of wastewater and solid waste generated during the manufacturing processes. It is evident from the table that both water consumption (300.0 m^3) and wastewater generation (250.0 m^3) is more in paper & pulp industries whereas the pollution load is more in distillery industry (600 kg of BOD).

Table 2.1 Water consumption, wastewater generation and pollution load in various Industries

S.No.	Industry	Water Consumption (m^3)	Wastewater generation (m^3)	Pollution load (kg of BOD)
1.	Dairy (Integrated) (per kilo litre of milk)	8.7	6	11
2.	Edible Oils and Vanaspati (per tonne oil)	3	2	7.5
3.	Fermentation - Brewery (per kilo Litre of beer) - Distillery (per kilo Litre of alcohol) - Maltry (per tonne of grain)	 11.5 130 8.5	 9.5 90 3.5	 24 600 2
4.	Starch (Maize Products) (per tonne of maize)	8	5.5	44
5.	Sugar (per tonne of cane crushed)	2	0.4	0.5
6.	Pulp & Paper (per tonne of Paper)	300	250	375

Source:-ENVIS, 1994.

2.3 Wastewater Discharge Standards

Wastewater discharge standards for nitrogenous fertilizer Industry was evaluated on the basis of technology used for pollution control in industry, end on treatment system and achievable quality of effluent by the technology which is in practice today (Environment Protection Rules, 1986). Table 2.2 summarizes the wastewater discharge standards for the nitrogenous fertilizer industry. Heavy metals like arsenic, vanadium and cyanide in wastewater must be very low in order to prevent its hazardous impact on the environment.

Table 2.2 Wastewater Discharge Standards for fertilizer industry

Parameter	**Plants Commissioned**	
	January 1, 1982 onwards	Prior to January 1, 1982
pH	6.5 to 8.0	6.5 to 8.0
Ammonical Nitrogen	50	75
Total Kjeldahl Nitrogen (TKN)	100	150
Free Ammonical nitrogen	4	4
Nitrate nitrogen	10	10
Cyanide	0.2	0.2
Vanadium	0.2	0.2
Arsenic	0.2	0.2
Suspended Solids	100	100
Oil & grease	10	10
Hexavalent chromium	0.1	0.1
Total chromium	2.0	2.0

Concentration not to exceed, 0.1 mg/L (except for pH)

Source: Environment Protection Rules, 1986.

2.4 Wastewater treatment methods and their evaluation

Discharge of wastewater by industries into natural water bodies depleted the dissolved oxygen to the level that it does not support aquatic life. This enforced the industries to implement suitable wastewater treatment technology. Industries adopted several physico-chemical and biological treatment processes. Physical treatment processes were done for removing large floating and suspended matter so as to protect main processes from possible damage (Kumar and Singh, 2009). It includes sedimentation, screening, grit removal, filtration etc. In chemical methods certain chemical reactions were used to improve the water quality. Chlorination is one of the most commonly used chemical methods. In neutralization, an acid or base is added for adjusting pH levels back to neutrality. Other methods include chemical precipitation, adsorption, ozonation and ion exchange. Adsorption involved adhesion of molecules to internal walls of pores in carbon particles produced by thermal activation (USEPA, 2000). Inactivation of pathogenic organisms was done by using ozone molecule to prevent waterborne diseases in the process of ozonation. Due to the capability of ozone to decompose into elemental oxygen in a short span of time, it was generated onsite (USEPA, 1999). However, biological methods were also involved in wastewater treatment where the micro-organisms were used for the biochemical decomposition of wastewater. It may be aerobic (activated sludge treatment methods, trickling filtration, oxidation ponds, lagoons, aerobic digestion) or anaerobic (anaerobic digestion, septic tanks, lagoons) methods. Despite the presence of these technologies, their application is limited due to certain drawbacks. The outline assessment of various wastewater treatment processes is given in Table 2.3.

Table 2.3 Merits and Demerits of wastewater treatment processes

S.No.	Treatment employed	Methods Used	Merits	Demerits
1.	Physical	Screening	• Appropriate for smaller plants with less screening. • Lower labor costs because of mechanically cleaning of screens.	• High equipment maintenance costs for mechanically cleaned screens.
		Grit Removal	• Removal of low putrescible organic content with a well-controlled aeration rate. • Improves the performance of downstream units by pre-aeration to reduce septic conditions in incoming wastewater.	• Release of potentially harmful volatile organics and odors from the aerated grit chamber. • Additional labor requirements for maintenance and control of aeration system.
		Sedimentation	• Reduction in organic and inorganic solids. • Less expensive method. • Simple in operation.	• Odor generation. • Moderate - large area requirements.
		Filtration	• Easy to operate. • Low cost.	• Requirement of mechanical devices.

		Flotation	• Suitable for removal of oil, grease and suspended solids.	• Initial equipment cost is high. • High maintenance cost.
		Equalization	• Dampers waste variations. • No requirement of chemicals.	• Requirement of large land area. • Requirement of mixing and aeration equipment and hence high capital and operation cost.
2.	Chemical	Chlorination	• Effective method for treatment of domestic wastewater. • Control of odors during disinfection. • Cost effective method. • Foam control. • Effective against wide spectrum of pathogenic micro-organisms.	• Safe handling requirements. • Production of toxic disinfection by-products. • Increase in levels of total dissolved solids in treated wastewater. • Oxidation of certain types of organic matter, creating more harmful compounds (e.g., trihalomethanes).
		Ozonation	• Best disinfection method. • Efficient in killing bacteria, viruses and protozoa. • No production of disinfectant by-products. • Elevates the dissolved oxygen concentration of wastewater.	• More complex technology in comparison to chlorination. • High capital cost. • Requirement of consistent monitoring because of toxicity of ozone. • Not economical for wastewater containing high levels of suspended solids, biochemical oxygen demand,

				chemical oxygen demand and total organic carbon.
		Neutralization	• For neutralizing wastewaters so as to prevent the damage caused by direct disposal of wastewater to the environment. • Reduction in corrosion and scaling.	• Sophisticated instrument requirement. • Generation of solids.
		Chemical precipitation	• Ready availability of chemicals and equipment. • Self-operating and low maintenance requirements. • Removal of metals and other suspended solids, inorganic solids, fats, oils and some organic substances from wastewater.	• Certain coagulants used in the process may add solids to wastewater. • Require operator safety as it involves corrosive chemicals.
		Adsorption	• Used for tertiary treatment of municipal and industrial wastewater. • Used for removal of dissolved organics from wastewater. • Less space requirements.	• Granular carbon beds may generate hydrogen sulphide from bacterial growth under certain conditions thereby creating odors and corrosion problems. • Requires pre-treated wastewater with low concentration of suspended solids.
		Ion exchange	• Low running cost. • Little energy requirements.	• Lack of selectivity against specific target ion.

			• Removal of ammonium ion from the wastewater. • Facility of regenerating ion exchanger.	• Susceptibility to fouling by organic substances present in wastewater.
3.	Biological	Trickling Filters	• Simple and reliable process suitable in areas where large land area is unavailable. • Used for organic matter removal from wastewater. • Suitable for reducing BOD_5 in applied wastewater. • Low power requirements.	• Due to lack of high surface area and high void age, these are less suitable for treatment of large volumes of strong industrial wastewaters. • Additional treatment of wastewater is needed to meet discharge standards. • High incidence of clogging.
		Activated Sludge	• Most efficient method of wastewater treatment. • Capable of removing 99% BOD and 97% suspended solids.	• High aeration cost. • High land requirement • Technically skilled manpower requirement for operation and maintenance.
		Oxidation Ponds	• Ease in operation and maintenance. • Effective wastewater treatment method with minimal threat to environment.	• Requires large area of land for construction. • Produce odors.
		Vertical Biological	• Most effective treatment method.	• High cost and energy requirement. • Technically skilled manpower

		Reactors	• Small land area requirement.	requirement for operation and maintenance.
		Septic Tanks	• Operation and maintenance is easy.	• Low treatment efficiency. • Requirement of landfill for proper disposal of sludge.
		Aerobic Digestion	• Simple and more efficient method for breaking down waste products. • Used in treatment of municipal wastewaters. • No odors production.	• High energy consumption. • Could not thicken sludge easily.
		Anaerobic Digestion	• Used in treatment of high strength wastewaters. • Less energy requirement. • Methane production. • Less biological sludge production.	• High capital cost. • Require further treatment to meet discharge requirements. • Production of odors and corrosive gases.

From the table, it is observed that the chemical methods in general add further pollutants to the environment despite their ability to provide quick results. The biological treatment processes involves large land requirement; high construction cost, high operation & maintenance cost, requirement of skilled workers for recurrent inspections and constant maintenance. These drawbacks have necessitated searching some other cost effective method of wastewater management. Wastewater reuse is considered as the most reliable, less costly methods of water supply and one of the best approaches for proper management of water resources and recycling wastewater. Irrigation with wastewater serves as an economic source for agriculture due to its nutrient richness and is seen as a way of preventing pollution of water bodies.

2.5 Effect of wastewater on irrigation

Although, wide range of treatment methods are employed for treating wastewater, but installation of a treatment plant is tedious as it involves a high cost and installation of sophisticated equipments which entails a huge area. For this reason, usage of wastewater for irrigation is considered as one of the best option to tackle the problem of industrial waste generation.

Since, the wastewater from fertilizer industry contains heavy metals like arsenic, chromium, cadmium, and vanadium etc., their discharge directly into water bodies would prove to be dangerous to the aquatic life. It was reported that fertilizer industry effluent affects the protein and activity of lactate dehydrogenase in different organs of freshwater teleost fish, *Channa striatus* (Yadav *et al.,* 2007).

Physicochemical analysis of selected fertilizer industry effluent and its effect on crop plants like wheat, barley, peas, spinach, fenugreek, coriander, cabbages, and turnips was studied by Ahmad *et al.*, (2000).

The literature regarding influence of different wastewaters on crops is reviewed and summarized in Table 2.4. It was found that the effect of wastewater on plant growth varies with different type of wastewater and different crops under different environmental conditions. From the table it is evident that several studies have been carried out with different types of wastewater ranging from agrochemical wastewater (distillery, dairy, sugar etc.) to highly toxic industrial wastewater (textile, tannery etc.) to evaluate its suitability for their use in agriculture (Hussain *et al.*, 1982; Singh and Mishra, 1987; Juwarkar and Dutta, 1989; Ramana *et al.,* 2002; Dhanam, 2009; Nath *et al.*, 2009 and Malaviya and Sharma, 2011). The wastewater from these industries could be used for raising crops only after appropriate dilution (mostly 25%) which again varied with the crop. The effect was more appropriate at lower dilution level of wastewater (Singh and Mishra, 1987; Sundaramoorthy *et al.*, 2001; Ramana *et al.*, 2002; Sukanya and Meli, 2004 and Dhanam, 2009). Higher concentration of wastewater showed inhibitory effects on the plant growth in terms of reduced seedling growth (Sundaramoorthy *et al.*, 2001; Singh *et al.*, 2006; Baghel, 2008 and Wins and Murugan, 2010) decreased germination rate (Singh and Mishra, 1987; Singh *et al.*, 2006 and Begum *et al.*, 2010), decreased chlorophyll content (Singh *et al.*, 2006 and Baghel, 2008), decreased fresh and dry weight (Giri, 2008 and Kamalakar *et. al.,* 1991), reduced carbohydrates and protein content (Misra and Behera, 1991 and Nath *et. al.,* 2009) and reduced enzyme activity (Nath *et. al.,* 2009).

2.6 Effect of wastewater on individual crops

The response of crops varies with respect to the type of wastewater used for irrigation. Table 2.5 summarizes the response of individual crops to different wastewaters. It is clear from the table that the wastewaters (distillery, fertilizer, monosodium glutamate, domestic sewage, biomass power plant, olive mill) have both positive impacts as well as negative impacts on crops. Positive impacts such as increased nitrogen accumulation in shoots (Singh *et al.*, 2009), increase in seedlings fresh weight (Ale *et al.*, 2008), increased plant height, leaf area, dry weight and crop growth rate (Aziz *et al.*, 1999), increased pod yield (Umebese *et al.*, 2009) and increase in chlorophyll a and b content (Nagajyothi *et al.*, 2009). The negative impacts such as burning of leaf tips and formation of loop by young emerging leaves, chlorotic and needle shaped leaves (Pandey *et al.*, 2008), leaf curling (Singh *et al.*, 2009), delayed flowering and fruiting (Bharagava *et al.*, 2008), decreased length of embryonic axis and root (Kannan and Upreti, 2008), inhibition in nitrogen fixation (Ramana *et al*., 2002), delayed seed germination (Bazai and Achakzai, 2006) and reduction in chlorophyll content (Singh *et al.*, 2006) was observed.

Table 2.4 Effect of different wastewaters on plant growth

S. No.	Type of wastewater	Author and year	Studies	Plant used	Outcomes
1.	**Fertilizer**	Singh and Mishra, 1987	Effect of fertilizer factory wastewater on soil and crop productivity.	Corn and rice	• Wastewater Concentrations used- 2.5%, 5% 10%, 25% and 50%. • Lower wastewater concentrations (2.5 and 5%) improved the growth and development of corn and rice whereas higher concentrations of wastewater (10% and above) inhibited the percentage of seed germination and caused detrimental effects on the dry matter production, yield (quantitative and qualitative) and the photosynthetic pigments of both the crops.
		Sundaramoorthy *et al.*, 2001	Effect of fertilizer factory effluent on germination and seedling growth of groundnut varieties.	groundnut varieties namely CO-2, ICGFDRI, TMV-2 and VRI-2	• Wastewater Concentrations used- 0%, 1%, 2%, 5%, 10%, 25%, 50%, 75% and 100%. • In all the four varieties, higher wastewater concentration of 25% and more, caused decline in the germination percentage and seedling growth. • VRI-2 variety was found to be more tolerant

					than all varieties studied. • 5% concentration favored for the better development of seedlings compared to other concentrations of wastewater.
		Singh *et al.*, 2006	Impact of fertilizer factory wastewater on seed germination, seedling growth and chlorophyll content of gram (*Cicer aeritenum).*	Gram	• 10%, 25%, 50%, 75% and 100% of wastewater concentrations were used for the study. • A progressive decline in seed germination percentage, seedling growth and chlorophyll content with increase in wastewater concentration beyond 25% was noted. • Toxic effects were observed at higher wastewater concentration of 50% and above.
		Adoki and Orugbani, 2007	Influence of nitrogenous fertilizer plant wastewaters on growth of selected farm crops in soils polluted with crude petroleum hydrocarbons.	Fluted pumpkin, maize and okra	• Nitrogen fertilizer plant wastewaters caused disappearance of crude oil in treated plots. • Good soil recovery was seen with crops grown on the experimental plots. • The study revealed that fertilizer factory wastewater can be applied to petroleum hydrocarbon contaminated soils in a controlled manner so as to improve crop

					germination recovery on such soils.
		Begum *et. al.*, 2010	Effect of industrial wastewaters on the germination and seedling growth of three leafy vegetables.	Red amaranth, amaranthus stem, mustard and radish	• Wastewater Concentrations used-control, 25%, 50%, 75% and 100%. • Increase in wastewater concentration resulted in reduction in relative germination ratio and root length but caused increase in shoot length.
2.	**Distillery**	Juwarkar and Dutta, 1989	Impact of distillery wastewater application to land on soil microflora (bacteria, fungi and actinomycetes)	-	• Reduced growth rates of Rhizobium and Azotobacter after application of raw wastewater. • Minimization in toxic effect on mixing with stabilization pond wastewater (1:1). • No fruits production on irrigation of groundnut plant with raw distillery wastewater.
		Ramana *et. al.*, 2002	Effect of Distillery wastewater on some physiological aspects in maize.	maize	• Leaf area, chlorophyll content, nitrate reductase activity, total dry weight and grain yield was increased with biomethanated and raw spent wash as compared to control.
		Ramana *et. al.*, 2002	Effect of Distillery wastewater on seed	tomato, chilli, bottle gourd,	• No inhibitory effect on seed germination at low concentration except in tomato.

			germination in some vegetable crops.	cucumber and onion	• On the basis of the tolerance of crops to the distillery wastewater, the crops studied were arranged in the following order cucumber>chilli>onion>bottle gourd>tomato.
		Singh *et. al.,* 2003	Effect of distillery wastewaters on plant and soil enzymatic activities and groundnut quality.	groundnut	• Biomethanated Spent Wash was superior in increasing the yield and nutritional quality parameters of groundnut. • Dehydrogenase and alkaline phosphatase activities were higher in BSW.
		Sukanya and Meli, 2004	Distillery wastewater effect on growth, yield and quality of maize	maize	• Lower dilution level of 1:5 showed significantly high grain yield. • Protein and reducing sugar contents of seed significantly higher in 1:5 dilutions.
		Kaushik *et. al.,* 2005	Impact of long and short term irrigation of a sodic soil with distillery wastewater in combination with bioamendments.	Pearl millet	• Long term application of PME (Post Methanation Effluent) in the field (10 years) significantly increased the Total organic carbon, Total kjeldahl nitrogen, available phosphorus, exchangeable potassium and soil enzymatic activities in the soil but build up harmful concentration of Na which could be chelated by bioamendments.

					• In short term studies in the laboratory (30 days), application of 50% PME along with bioamendments proved to be most useful in improving the properties of sodic soil and also favoured successful germination and improved seedling growth of pearl millet.
		Pandey *et. al.*, 2007	Physico-chemical analysis and effect of Distillery wastewater on seed germination.	Wheat, *Pea and Lady's Finger.*	• Germination percentage decreased with increased wastewater concentrations from control to 100%. • Germination speed, peak value and germination value were increased from control to 25% and 50% and decreased from 50% to 75% and 100% wastewater.
		Baghel, 2008	Toxicity of distillery wastewater on seed germination, seedling growth and metabolism in plant.	Pea	• Seed germination and seedling growth were significantly reduced with increase in concentration of distillery wastewater. • Seedling growth (radicle and plumule size), number of lateral roots, total chlorophyll, total amylase, fresh weight, dry weight and moisture content were adversely affected.
		Malaviya and	Impact of distillery	*Brassica*	• Wastewater Concentrations used-0%, 20%,

		Sharma, 2011	effluent on germination behaviour of *Brassica napus* L.	*napus* L. var. Punjabi Special	40%, 60%, 80% and 100%. • High values of percent germination, germination index, speed of germination and peak values and low values of percent inhibition, germination period and delay index in 20% of wastewater concentration were recorded..
		Rath *et. al.,* 2011	Effect of distillery spent wash and fertilizer on growth and chlorophyll content of sugarcane plant.	Sugarcane	• Growth parameters (height of the plant, length and girth of stem, breadth of leaves, number of leaves and number of tillers per plant, leaf area index and total chlorophyll content of sugarcane plant increased in fields treated with inorganic fertilizer, inorganic fertilizer and cow dung and in 50% of distillery spent wash over control whereas in 100% distillery spent wash, a declining trend in all parameters over the control was observed. • Maximum growth and chlorophyll content was observed in 50% distillery spent wash in comparison to the fields treated with two different types of fertilizers (inorganic fertilizer

					and cow dung).
3.	**Dairy**	Dhanam, 2009	Effect of dairy wastewater on seed germination, seedling growth and biochemical parameter in paddy.	Paddy variety ADT-38	• Various concentrations of dairy wastewaters used- 0% (control), 5%, 10%, 25%, 50%, 75% and 100% • At lower dilutions (25%), favourable effect on seed germination, seedling growth and dry matter production and biochemical parameters (protein, starch, amino acid and pigment content) over control was observed. • 100% wastewater concentration caused inhibitory effect.
		Gaikar *et. al.*, 2010	Effect of dairy wastewater on seed germination and seedling growth of soyabeans.	Soyabean	• Various concentration-control, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% were used. • Germination percentage decreased with increasing wastewater concentration. • Seedling growth gradually increased up to 50% wastewater concentration. • 10% dilution of wastewater improved seed germination and 100% inhibited both seed germination and seedling growth.

					• The dairy wastewater could be used as liquid fertilizer up to 50% dilution.
4.	**Paper and Pulp**	Hussain *et. al.*, 1982	Effect of wastewaters on germination and growth of some cultivated plants.	*Brassica campestris, Lens culinaris, Triticum vulgare, Hordeum vulgare, Zea mays, Trifolium resupinatum, Cicer arietinum* and *Pennisetum americanum*	• Wastewater Concentrations used-control, 25%, 50% and 100%. • *Zea* showed 17% increase in germination followed by *Brassica* and *Triticum*, radicle growth of *Brassica* and *Triticum* both of which showed 100% increase, plumule growth of *Triticum* and *Zea* both showed 200% increase and lateral roots of *Zea* exhibited 87% increase followed by *Hordeum* with 33% increase against their controls. • In pot experiments, number of leaves, height of shoots, length of roots, fresh and dry mass, moisture contents of *Hordeum* shoots was better than that of controls. • In field experiments, number of leaves, height of shoots, length of roots, fresh and dry mass of shoots and roots significantly increased under test conditions. Moisture contents of shoots and roots of *Brassica* and *Hordeum* and roots of *Triticum* were slightly decreased

					while remaining species had either equal or slightly better moisture contents than their controls.
		Misra and Behera, 1991	The effect of paper industry wastewater on growth, pigments, carbohydrates and proteins of rice seedlings.	*Oryza sativa* L. cv. Kesari-82K	• Decrease in percentage of germination, water imbibing capacity, growth, pigment, carbohydrate and protein content with increase in wastewater concentration and time.
		Kamalakar *et. al.,* 1991	Effect of paper and pulp factory wastewaters on the growth and development of maize and sunflower.	Maize and sunflower	• Germination and vigour index of seedlings were poor at higher concentrations of wastewater. • Plant height, mean fresh weight of shoot and root decreased with increased wastewater concentrations. • Wastewater at lower concentration (25%) had significant effect on seed germination, growth and development of seedlings of both maize and sunflower.
		Dane *et. al.,* 2006	The effect of wastewater on root	Onion	• Low concentrations of minerals in waters of effluent channels had some positive effects

			growth and mitosis in onion root apical meristem.		on root growth and mitotic divisions in onion root tip cells.
		Giri, 2008	Effect of paper and pulp mill wastewater on germination, growth, biochemical parameters and bioaccumulation of heavy metal in *Pisum sativum*.	*Pisum sativum*	• Different wastewater concentrations used- 0%, 10%, 20%, 50% and 100%. • Maximum fresh weight, dry weight and chlorophyll contents were observed at 50% of wastewater concentration but protein contents was found to be maximum in 25% concentration of wastewater. • 100% wastewater concentration showed retardation of growth. • Increase in value of peroxidase and catalase with increase in concentration of wastewater • Acid phosphatase value increased with increase in concentration till 50% of treated wastewater whereas ribonuclease value decreased significantly with increase in concentration in 25% but increases in 50% and 100% of wastewater concentrations.
		Lin *et. al.,* 2008	Application of	-	• Wastewater use-Paper industry wastewater

			wastewater from paper and food seasoning industries with green manure to increase soil organic carbon.		(WP), food industry (WS) and a combination of WP+WS. • In comparison to control, increase in alkali soluble organic carbon fraction by 59% in the soils supplied with green manure containing WS and by 31% in the treatment without green manure was noted. • A 63% increase in organic carbon was observed in the soil supplied with paper mill wastewater. • 90% increase in alkali-soluble organic carbon fraction in the soil was seen with the combined treatment of WP+WS containing green manure as compared to control whereas in the treatment without green manure, a 71% increase in organic carbon was noted.
		Medhi *et. al.*, 2011	Impact of paper mill wastewater on growth and development of certain agricultural crops.	rice, mustard, pea	• Wastewater Concentrations used-10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 100% • At higher concentration, paper mill wastewater has deleterious effect on crops growth.

					• At lower concentrations (viz. 10%-40% in rice, 10%-50% in mustard and 10%-60% in pea) beneficial effect on general welfare of crops was observed. • Growth and development of rice, mustard and pea was increased with increase in concentration up to 30%, 40% and 50% wastewater, respectively.
5.	**Tannery**	Nath *et. al.,* 2009	Amelioration of treated tannery wastewater toxicity in radish based on nutrient application.	*Raphanus sativus*	• Wastewater Concentrations used- control, 10%, 25%, 50% and 100%. • 10 and 25ppm of certain macro (potassium) and micro nutrient (iron and zinc) were used in the form of zinc sulphate, potassium sulphate and iron sulphate and added in 50% tannery wastewater. • At 25% concentration, increase in leaf area, fresh and dry weights was observed. • Decrease in chlorophyll, pheophytin, carotenoids, protein and sugar content was examined with the increase in wastewater concentration from control to 100%. • Significant increase in catalase and

					peroxidase activity was observed with increasing wastewater concentration up to 50%.
		Tayyar and Yapici, 2009	Seed germination characteristics of crops irrigated with different dilutions of pre-tanning wastewaters.	Broad bean, lentil and common bean	• Wastewater concentrations used-control, 1:10, 1:40 and 1:80. • Absence of seed germination was seen on irrigation with undiluted pre-tanning wastewaters. • Inhibition in seed germination decreased with the decrease in concentration of wastewater.
		Hussain *et. al.,* 2010	Effect of tannery wastewaters on seed germination and growth of two sunflower cultivars.	Sunflower cultivars-FH-330 and FH-245.	• Wastewater Concentrations used- 0%, 20%, 40%, 60%, 80% and 100%. • Plant height, number of leaves per plant, percentage of germination, chlorophyll, proteins and carbohydrates content of both the sunflower cultivars was decreased with the increase in wastewater concentrations from 0% to 100%. • Decrease in yield of both sunflower cultivars was significantly reduced with increase in wastewater concentration.

					• Appearance of pale yellow color of the affected leaves was observed under higher wastewater concentrations. • Reduction in biomass accumulation and reproductive growth was seen in 100% wastewater concentration.
6.	**Textile**	Nawaz *et. al.*, 2006	Effect of industrial wastewaters on seed germination and early growth of *Cicer arientum*.	*Cicer arientum* - P-91 and P-2000	• Wastewaters used-textile mill wastewater, marble industry and refinery wastewater. • Wastewater Concentrations used- 0%, 10%, 20%, 40%, 80% and 100%. • With the increase in wastewater concentrations, growth of plants was more affected in textile mill wastewater in comparison to marble industry and refinery wastewaters. • Fresh weight was less in textile mill wastewater in comparison to control whereas in other two wastewaters both increase and decrease in dry weights were observed.
		Garg and Kaushik, 2008	Influence of textile mill wastewater irrigation on	Pioneer jowar and	• Wastewater Concentrations used- 0%, 6.25%, 12.5%, 25%, 50%, 75% and 100%.

			the growth of sorghum cultivars.	Desi jowar	• No inhibitory effect on seed germination was seen at lower (6.25%) wastewater concentration. • Although, seeds were germinated in 100% of textile wastewater but did not survive for longer periods.
		Wins and Murugan, 2010	Effect of textile mill wastewater on growth and germination of Black gram.	*Vigna mungo* (L.) *Hepper*	• Wastewater Concentrations used- control, 25%, 50%, 75% and 100%. • Germination ratio and growth were higher in 25% wastewater concentration. • It was found that root and shoot length was decreased beyond 25%.
7.	**Sugar**	Doke *et. al.,* 2011	Physico-chemical analysis of sugar industry wastewater and its effect on seed germination of crops.	*Vigna angularis*, *Vigna cylindrical* and *Sorghum cernum*	• Wastewater Concentrations used- 0%, 20%, 40%, 60%, 80% and 100%. • Decrease in germination percentage and germination values was recorded with increasing wastewater concentration.

Table 2.5 Crop specific effect of wastewater

Plant Studied	Wastewater Used	Impacts	Reference
Maize	Distillery	Leaf tips burning, loop formation by young emerging leaves and marked decrease in leaf size was observed with 100% wastewater.	Pandey *et al.*, 2008
	Monosodium glutamate	Increased nitrogen accumulation in shoots and significantly higher biomass with the increase in wastewater concentrations.	Singh *et al.*, 2009
	Petrochemical industry	Plant height, leaf area, fresh weight, dry weight, crop growth rate was increased with treated wastewater irrigation in comparison to irrigation with fresh water.	Aziz *et al.*, 1999
Rice	Distillery	Fresh weight of seedlings was increased at lower concentrations (1% and 5%) of wastewater.	Ale *et al.*, 2008
		Leaves of rice seedlings failed to unroll and remain needle-like, their apical part turned chlorotic, root tips turned brown and necrotic on irrigation with 100% wastewater.	Pandey *et al.*, 2008
Chinese cabbage	Monosodium glutamate	Mild symptoms such as curling of Chinese cabbage leaf in MSGW-15 treatment were seen.	Singh *et al.*, 2009

Wheat	Monosodium glutamate	Linear relationship existed between COD_{cr} concentration in monosodium glutamate wastewater and inhibition rate of seed germination and root elongation of wheat.	Rui *et al.*, 2007
Cicer aeritenum	Fertilizer	Significant fall in chlorophyll content under higher wastewater concentration (>25%) was recorded.	Singh *et al.*, 2006
Mustard	Post methanated distillery effluent	Initially, vigorous growth was observed but later reduced growth, delay in flowering and fruiting was seen in comparison to control.	Bharagava *et al.*, 2008
Top Vegetables Creepers	Distillery Spent wash	Yields of all vegetables were high in 33% of spent wash, moderate in 50% spent wash concentration and reduced in raw water. Plants were able to absorb nutrients from soil as well as from the spent wash thereby increasing yields in 33% spent wash.	Chidankumar *et al.*, 2009
Mung beans	Distillery	Length of embryonic axis and root was significantly reduced on pre-soaking of seeds in untreated wastewater for 6 hours.	Kannan and Upreti, 2008
Cow pea	Combined effluent	Growth and pod yield of red cultivar and pod yield of white cultivar of cow pea was enhanced with the direct application of the combined effluent.	Umebese *et al.*, 2009
Groundnut	Distillery spent wash	Nitrogen fixation in groundnut was inhibited on irrigation with raw spent wash.	Ramana *et al*., 2002

Tomato	Monosodium glutamate	Tomato was sensitive to monosodium glutamate wastewater and thus considered as an ideal toxic bioindicator.	Rui *et al.*, 2007
Lettuce	Domestic sewage	Delay in seed germination was noticed with the increase in wastewater concentration.	Bazai and Achakzai, 2006
Green gram	Biomass power plant	Increase in root and shoot length, germination percentage, chlorophyll a, b, and total content was recorded at 25% wastewater concentration.	Nagajyothi *et al.*, 2009
Barley	Olive mill wastewater	Necrotic spots on the leaves and reduction in secondary stem emergence was observed with the usage of wastewater without pretreatment during tillering stage.	Rousan, 2007
Cotton	Distillery spent wash	Sprouting of seeds did not occur in 1:1 spent wash.	Chandraju *et al.*, 2011

2.7 Summary of literature review

This chapter provides an overview of the volume of wastewater generated by different industries, discharge standards for the nitrogenous fertilizer industry, various wastewater treatment methods along with their benefits and shortcomings. The effect of both treated as well as untreated wastewaters on various agronomic crops was also reviewed. It can be concluded that the wastewaters have both favorable as well as adverse effect on crops. Since, no study has done yet on effect of fertilizer industry wastewater on barley; it is interesting to know the response of barley towards fertilizer industry wastewater.

CHAPTER 3

OBJECTIVES

The experimental set up was designed and conducted in Environmental Science lab, Central University of Punjab, Bathinda. The objectives of the present study were

- To collect and characterize the fertilizer industry wastewater
- To study the effect of fertilizer industry wastewater on the germination behavior of barley grains (*Hordeum vulgare).*
- To assess the effect on germination by
    a) Morphological (germination percentage, germination index and germination vigor index, root and shoot length, fresh and dry weight, relative elongation ratio of shoot and root, chlorophyll a, b and total chlorophyll content) and
    b) Biochemical parameters (α amylase, protease, peroxidase, superoxide dismutase, proteins and carbohydrates) of germinated grains.

The detailed methodology followed in the present study is provided in Figure 3.1.

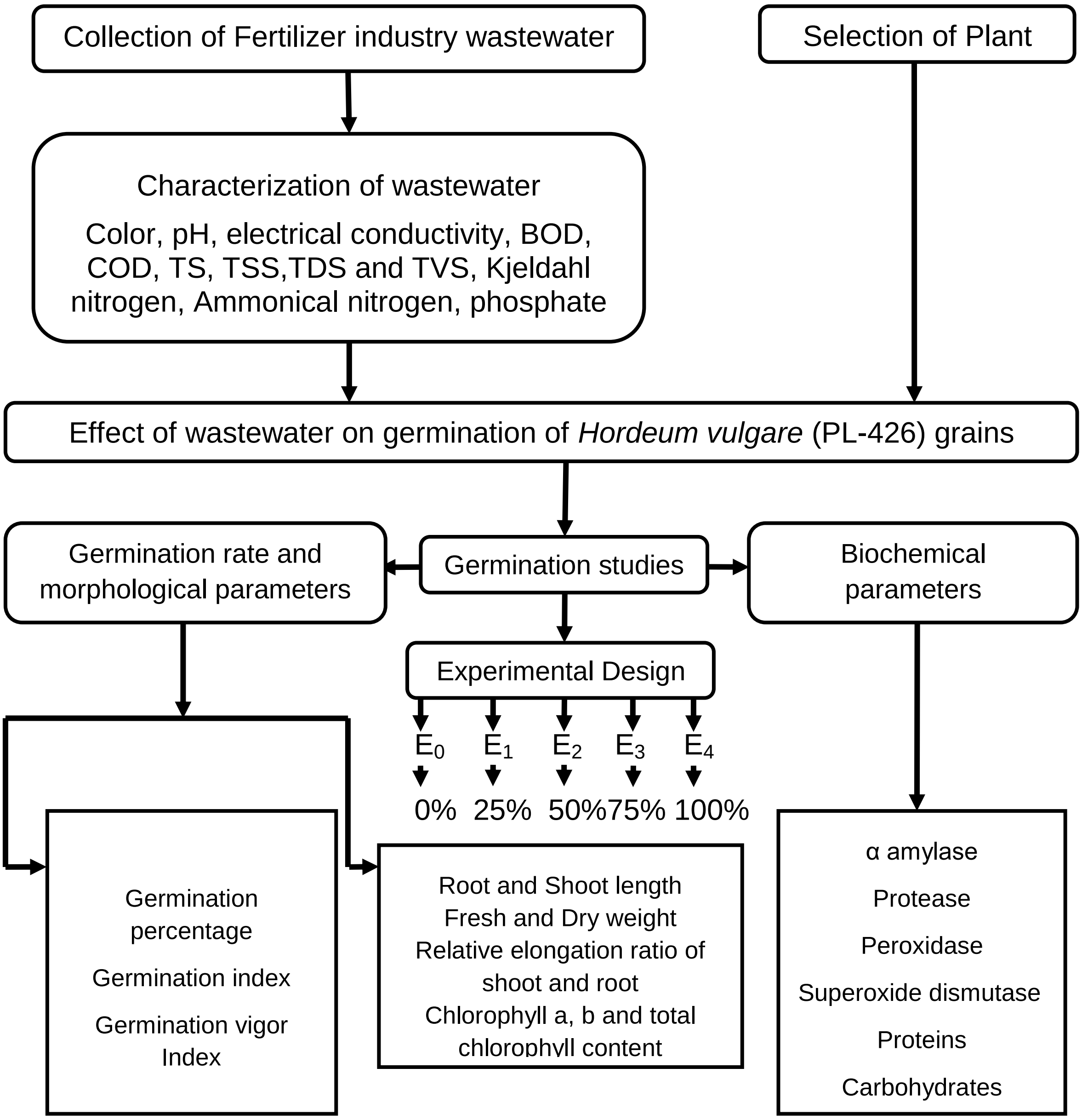

Figure 3.1 Outline of Methodology

CHAPTER 4

MATERIALS AND METHODS

4.1 Collection of Wastewater

The wastewater used for the present study was collected from National Fertilizer Limited, Bathinda in cans of capacity 10 L. The industry consisted of two plants- ammonia and urea plant and had started its commercial production on 01-10-1979. Approximately 50 L of untreated wastewater was collected from the ammonia plant, in cans of 10L capacity and preserved in cold rooms (1-5°C) at Central University of Punjab, Bathinda. The collected wastewater was then analysed for various physico-chemical parameters.

Figure 4.1 (a) Wastewater flowing out of pipelines, (b) Close view and (c) Wastewater Collection

4.2 Physico-chemical characterization of wastewater

The fertilizer industry wastewater was analysed for various physico-chemical parameters as listed in Table 4.1. The characterization of wastewater was done according to Standard methods for water and wastewater examination (APHA, 2005).

Table 4.1 Physico-chemical parameters

S.No.	Physico-chemical parameters	Methods employed	Reference
1.	pH	Water Analysing Kit	-
2.	Electrical conductivity	Water Analysing Kit	-
3.	Biochemical Oxygen Demand	Azide modification	APHA 5210 B, 5-2
4.	Chemical Oxygen Demand	Open Reflux	APHA 5220 B, 5-15
5.	Total Solids	Gravimetric	APHA 2540 B, 2-56
6.	Total Suspended Solids	Gravimetric	APHA 2540 B, 2-58
7.	Total Dissolved Solids	Gravimetric	APHA 2540 B, 2-57
8.	Total Volatile Solids	Gravimetric	APHA 2540 B, 2-59
9.	Total Kjeldahl Nitrogen	Kjeldahl	APHA 4500-B,4-131
10.	Total Ammonical Nitrogen	Kjeldahl	ISI: 3025 (Part 34)-1988, 5-6
11.	Phosphate	Stannous Chloride	APHA 4500-D, 4-152

4.3 Effect of fertilizer industry wastewater on germination of *Hordeum vulgare* (PL-426) grains

4.3.1 Experimental set up

The experimental set up was designated as E_0, E_1, E_2, E_3 and E_4 based on the composition of wastewater used for the treatment (Table 4.2). Five treatments were carried out in triplicates. E_0 was taken as control which consisted of distilled water. For E_1, E_2, E_3, E_4, the wastewater concentration varied as 0% v/v, 25% v/v, 50% v/v, 75% v/v and 100% v/v, respectively.

4.3.2 Germination studies

Petriplates of 140 mm diameter were used for the germination study. The petriplates were lined with filter paper and designated as E_0, E_1, E_2, E_3 and $E_{4.}$ In each petriplate, around 15 seeds were placed with uniform spacing and treated with 6 ml of each concentration. The Emergence of radical and plumule were considered as a measure of germination. After 6 days, various morphological parameters and biochemical parameters were studied. The morphological parameters include root and shoot length, fresh and dry weight, germination percentage, relative elongation ratio of shoot and root, germination Index, germination vigor index and chlorophyll a, b and total chlorophyll content. The biochemical parameters include amylase, protease, peroxidise, superoxide dismutase, proteins and carbohydrates. Parameters like germination percentage, relative elongation ratio of shoot, relative elongation ratio of root, germination Index, germination vigor index and chlorophyll a and b are calculated according to the equations 4.1, 4.2, 4.3, 4.4, 4.5, 4.6 and 4.7, respectively.

Table 4.2 Concentration of Wastewater

Treatment	Distilled Water (ml)	Wastewater (ml)	Concentration (%)
E_0	6	0	0
E_1	4.5	1.5	25
E_2	3	3	50
E_3	1.5	4.5	75
E_4	0	6	100

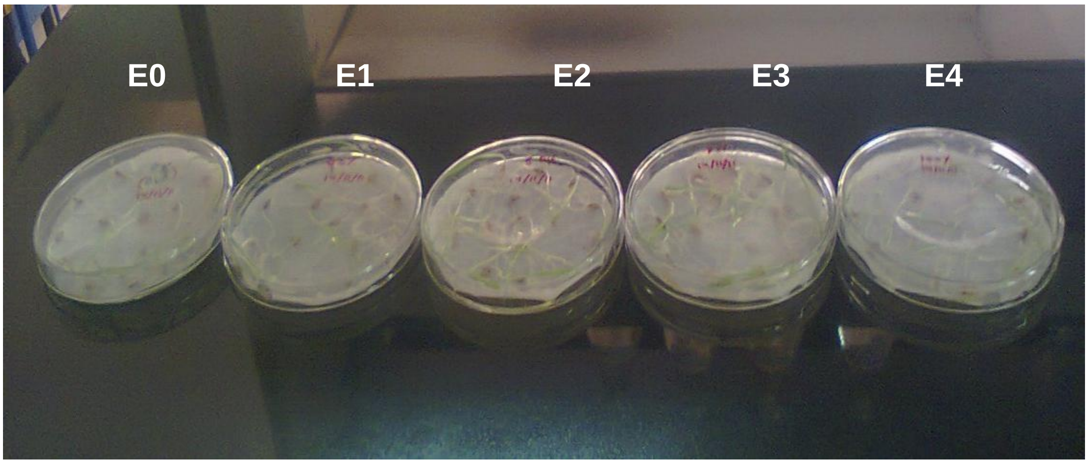

Figure 4.2 A photograph showing the experimental set up of germination study E_0 = Control, E_1 = 25% v/v wastewater concentration, E_2 = 50% v/v wastewater concentration, E_3 = 75% v/v wastewater concentration and E_4 = 100% v/v wastewater concentration.

4.3.2.1 Analytical methods

All the analytical process was carried out using Standard methods for examination of water and wastewater (APHA, 2005). The Root and shoot length of *Hordeum vulgare* (PL-426) grains was measured with the help of meter scale. Fresh and dry weight of grains of barley was measured on a weighing balance. Roots and shoots were separated from the plant and fresh weight of biomass (Roots and Shoots) were measured with the help of weighing balance and expressed in grams. For dry weight measurements, grains were wrapped in labelled blotting papers, oven dried at 80° C for 24 hours. After 24 hours, dry weight was recorded on the Citizen, CX120 weighing balance.

4.3.2.1.1 Germination percentage

Germination percentage of plant grown in distilled water and in different concentration of wastewater can be calculated by using following equation:

$$\text{Germination percentage} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds taken for germination}} \times 100 \quad \cdots\cdots\cdots\cdots \quad \textbf{(4.1)}$$

4.3.2.1.2 Relative Elongation Ratio (RER) of shoot

Relative Elongation Ratio of shoot of *Hordeum vulgare* (PL-426) grains grown in distilled water and in different concentrations of wastewater can be calculated by using following equation:

$$\text{RER of shoot} = \frac{\text{Mean shoot length of tested plant}}{\text{Mean shoot length of control}} \times 100 \quad \cdots\cdots\cdots\cdots \quad \textbf{(4.2)}$$

4.3.2.1.3 Relative Elongation Ratio (RER) of root

Relative Elongation Ratio of root of *Hordeum vulgare* (PL-426) grains grown in distilled water and in different concentrations of wastewater can be calculated by using following equation:

$$\text{RER of root} = \frac{\text{Mean root length of tested plant}}{\text{Mean root length of control}} \times 100 \quad \cdots\cdots\cdots\cdots \quad \textbf{(4.3)}$$

4.3.2.1.4 Germination Index

Germinated Index was calculated as follows:

$$\text{Germination Index (GI)} = \frac{\text{RSG} \times \text{RRE}}{100} \quad \cdots\cdots\cdots\cdots \quad \textbf{(4.4)}$$

Where, RSG is Relative Seed Germination and RRE is Relative Root Elongation.

$$\text{Relative Seed Germination} = \frac{\text{Number of seeds germinated in Test}}{\text{Number of seeds germinated in Control}} \times 100$$

$$\text{Relative Root Elongation} = \frac{\text{Root length in Test}}{\text{Root length in Control}} \times 100$$

4.3.2.1.5 Germination vigor index

Germination vigor index was calculated according to Abdul-Baki and Anderson (1973).

$$\text{VI} = \text{Germination percentage (\%)} \times \text{Seedling total length} \quad \cdots\cdots\cdots\cdots \quad \textbf{(4.5)}$$

Where VI = Vigor Index

4.3.2.1.6 Determination of Total chlorophyll content (Hiscox and Israelstam, 1979)

The total chlorophyll was estimated by Hiscox and Israelstam (1979) method (1949). 50 mg of *Hordeum vulgare* (PL-426) leaves were taken and suspended in test tubes containing 8 ml of Dimethylsulfoxide. The test tubes were incubated at 60˚C for 1 hour. The chlorophyll thus recovered in DMSO was measured at dual wavelength of 645 nm and 663 nm on Shimadzn UV-190 Double beam spectrophotometer using DMSO as blank. Chlorophyll a and b were calculated using the following equations given by Arnon (1949):-

Calculations

The calculation for both chlorophyll a and b was done on fresh weight basis.

$$\text{Chla} = (10.63 \times A_{663}) - (2.39 \times A_{663}) = X \quad \cdots\cdots\cdots\cdots \quad \textbf{(4.6)}$$

$$\text{Chl b} = (20.11 \times A_{645}) - (5.18 \times A_{663}) = Y \quad \cdots\cdots\cdots\cdots \quad \textbf{(4.7)}$$

$$\text{Ratio of chl } \frac{a}{b} = \frac{X}{Y}$$

Total chlorophyll= chla+chl b

Where A_{645} and A_{663} represent extinction values at 645 nm and 663 nm respectively.

4.3.2.2 Biochemical parameters

4.3.2.2.1 Determination of α amylase activity (Muentz, 1977)

50 mg of roots were homogenized in 2 ml of 0.1M potassium phosphate buffer (pH 7.0) under ice cold conditions using pestle and mortar. Homogenate was then centrifuged at 15,000 g for 30 minutes. The supernatant was stored at 4˚C until used for assaying the enzymatic activities.

To the 0.5 ml of enzyme extract, 1 ml of substrate solution (150 mg soluble starch, 600 mg potassium dihydrogen phosphate and 20 mg of anhydrous calcium chloride in 100 ml of distilled water, boil for 1 min, cool and filtered) was added. The mixture was incubated for half an hour. Then 0.1 ml of 0.1 M EDTA was added. To 0.2 ml of reaction mixture, 3 ml of iodine solution (25.4 mg iodine dissolved with 0.4 g potassium iodide in 100 ml distilled water) was added. The concentration of left over starch was measured spectrophotometrically at 630 nm. A blank was prepared by addition of distilled water in place of enzyme extract. The values obtained were compared against a starch standard curve prepared. Amylase activity was expressed as µg min^{-1} mg^{-1} protein.

4.3.2.2.2 Determination of Protease activity (Basha and Beavers, 1975)

To the 0.5 ml of enzyme extract, 0.5 ml of casein solution was added and the mixture was incubated at 37˚ C for 1 hour. To precipitate the proteins, 1 ml of TCA solution was added to the above mixture. The contents were then centrifuged at 10,000 rpm for 5 minutes. The supernatant so obtained was then used for enzyme activity estimation by method of Lowry *et al.* (1951). Enzyme activity was calculated against tyrosine as standard and expressed as ug/h/mg protein.

4.3.2.2.3 Determination of Peroxidase activity (Shannon *et al.*, 1966)

The reaction mixture contained 3 ml of 0.05 M guaiacol in 0.1 M phosphate buffer (pH 6.5), 0.1 ml of enzyme extract and 0.1 ml of 0.8 M H_2O_2. Reaction mixture without H_2O_2 was measured as blank. The reaction was initiated through addition of H_2O_2 and rate of change of absorbance was recorded at 470 nm for 3 minutes at an interval of 30 seconds. Tetrahydroguaiacol was absorbed at 470 nm. Peroxidase activity has been calculated according to the equation 3.9 and defined as change in absorbance/min/g of tissue.

Calculations

$$\% \text{ inhibition} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

50% inhibition corresponds to one unit

X% inhibition corresponds to 1/50* X = Y unit

$$\text{Activity} = \frac{Y}{\text{Volume of enzyme taken for estimation (ml)}} \times \frac{\text{Total enzyme (ml)}}{\text{Fresh weight (g)}} \quad \text{.................. } \mathbf{(4.8)}$$

4.3.2.2.4 Determination of Superoxide Dismutase activity (Marklund and Marklund, 1974)

1.5 ml of 0.1M Tris-HCl buffer (pH 8.2), 0.5 ml of 6mM EDTA, 1 ml of 6mM pyrogallol solution and 0.1 ml of enzyme extract was added to the cuvette. Absorbance was recorded at 420 nm after an interval of 30 seconds up to 30 minutes. Superoxide dismutase activity was calculated as per equation 3.10. Unit of enzyme activity has been defined as the amount of enzyme causing 50% inhibition of auto-oxidation of pyrogallol observed in blank.

Calculations

$$\Delta A = \text{Change in absorbance} = \frac{\text{Final- 0}}{3}$$

$$\text{Activity} = \frac{\Delta A}{\text{Enzyme extract (ml)}} \times \frac{\text{Volume after centrifugation (ml)}}{\text{Fresh weight (g)}} \quad \text{..................} \quad \textbf{(4.9)}$$

4.3.2.2.5 Determination of Proteins (Lowry *et al.*, 1951)

To 0.5 ml of enzyme extract, 2.5 ml of reagent C (50 ml of reagent A mixed with 1 ml of reagent B) was added. Mixed the contents well and allowed to stand for 10 minutes at room temperature. Then 2.5 ml of reagent D (Folin Ciocalteau's reagent diluted with water in the ratio of 1:1) was added and mixed rapidly. After 30 minutes, the intensity of blue colour was read at 520 nm. Amount of protein was calculated from the standard curve prepared by taking Bovine Serum Albumin as standard.

4.3.2.2.6 Determination of Carbohydrates (Loewus, 1952)

Procedure

To 1 ml of extract, 4 ml of anthrone's reagent was added with continuous shaking. Material was heated at 100˚ C for 20 minutes. The colour changes from brown yellow to green. This colour change was measured spectrophotometrically at 620 nm. Standard curve was prepared using glucose.

4.4 Statistical analysis

There were at least three replicates of each parameter or experiment. Data were analyzed statistically and results were expressed as mean±S.D. One way Analysis Of Variance (ANOVA) was carried out in order to evaluate significant difference between different treatments. Tukey's HSD post hoc test was used using software package PASW Statistics 18. Differences were considered significant at $p<0.05$.

CHAPTER 5

RESULTS

5.1 Wastewater characterization

The physico-chemical characterization of fertilizer industry wastewater is given in Table 5.1.

Table 5.1 Physico-chemical characteristics of Fertilizer industry wastewater

S.No.	Physico-chemical parameters	Value
1.	Colour	Transparent
2.	pH	2.2
3.	Electrical conductivity (µS)	1.03
4.	Biochemical Oxygen Demand (mg/L)	60.68
5.	Chemical Oxygen Demand (mg/L)	560
6.	Total Solids (mg/L)	6495
7.	Total Suspended Solids (mg/L)	600
8.	Total Dissolved Solids (mg/L)	2440
9.	Total Volatile Solids (mg/L)	3300
10.	Total Kjeldahl Nitrogen (mg/L)	25.76
11.	Total Ammonical Nitrogen (mg/L)	24.64
12.	Phosphate (mg/L)	0.067

The fertilizer industry wastewater was transparent in colour and highly acidic in nature (pH 2.2). It contains 6495 mg/L of total solids out of which around 50.8% were total volatile solids, 37.56% was total dissolved solids and 9.24% was total suspended solids. The BOD and COD of the wastewater were 60.68 and 560 mg/L, respectively.

Ammonia nitrogen was the major contributor of nitrogen compound and was around 24.64 mg/L.

5.2 Effect of fertilizer industry wastewater on germination of *Hordeum vulgare* (PL-426) grains

The effects of fertilizer industry wastewater on germination of *Hordeum vulgare* (PL-426) grains were administered through germination rate and various morphological parameters and biochemical parameters.

5.2.1 Effect on germination rate

Germination rate i.e. germination percentage, relative germination ratio, germination index and germination vigor index of *Hordeum vulgare* (PL-426) grains were studied after 6 days of germination period. It was found that differences in germination rate in different concentrations (E_0, E_1, E_2, E_3 and E_4) were mostly statistically significant ($p<0.05$).

5.2.1.1 Germination percentage

Germination percentage of *Hordeum vulgare* (PL-426) grains in E_0 and in E_2 was 95.53%. Decrease in germination percentage was observed beyond 50% of wastewater concentration (Fig 5.1). A significant decline in germination percentage (84.47%) was recorded in E_4 when compared to control.

5.2.1.2 Germination index

The germination index when compared to control (E_0) was enhanced by 6.7% and 31.5% in E_1 and E_2, respectively. However at E_2, the increase was significant in comparison to that of control (Fig 5.2). In E_4, an insignificant decline by 16% was noted showing minimum germination index.

5.2.1.3 Germination vigor index

Germination vigor index responded significantly ($p<0.05$) to the different concentrations of wastewater (Fig 5.3). Maximum value was recorded at E_2

(1434.35). A significant decline in vigor index was observed at E_4 indicating minimum vigor index at E_4 (1026.29) in comparison to control.

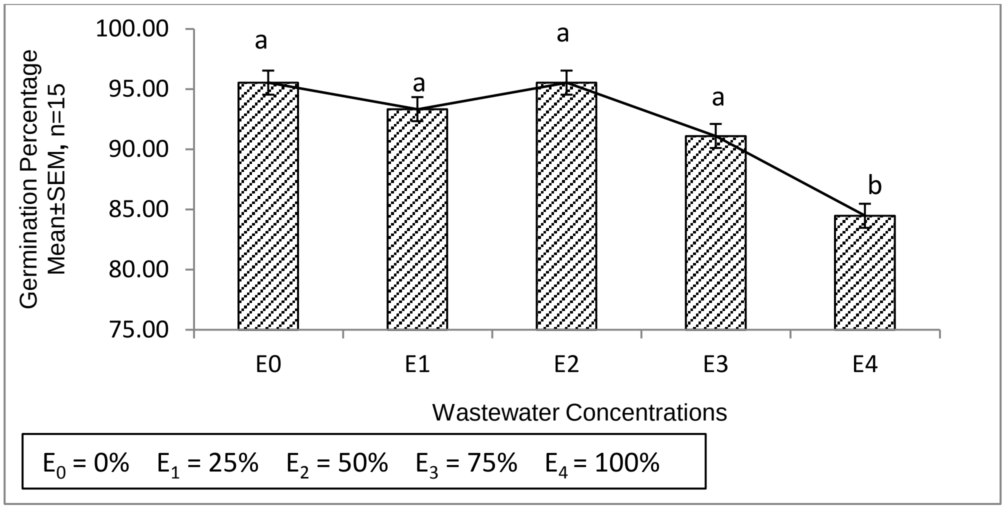

Figure 5.1 Variation in germination percentage of *Hordeum vulgare* (PL-426) grains at different wastewater concentrations. Data has been analyzed by one way ANOVA, post hoc multiple comparison Tukey's test. Data following same letter are not significantly different by Tukey's test at the 5% level.

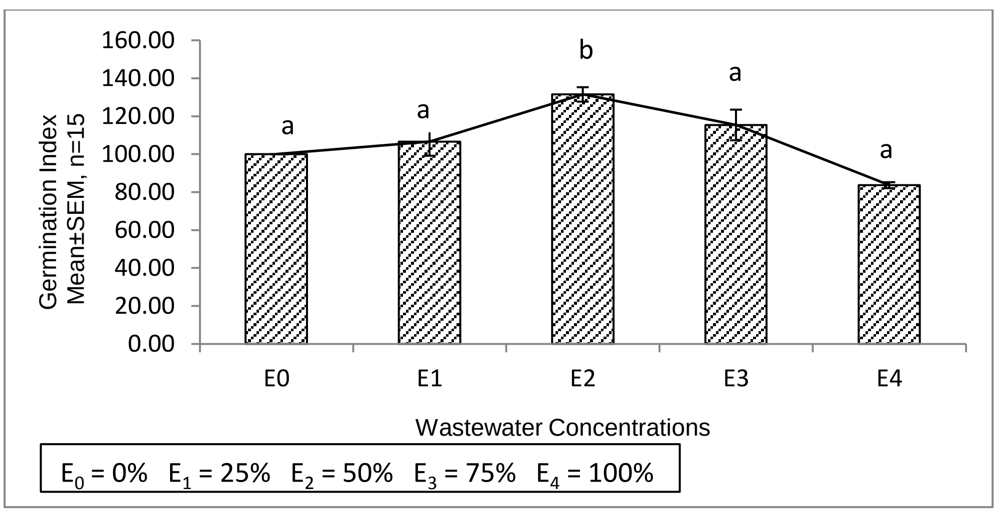

Figure 5.2 Variation in germination index of *Hordeum vulgare* (PL-426) grains at different wastewater concentrations. Data has been analyzed by one way ANOVA, post hoc multiple comparison Tukey's test. Data following same letter are not significantly different by Tukey's test at the 5% level.

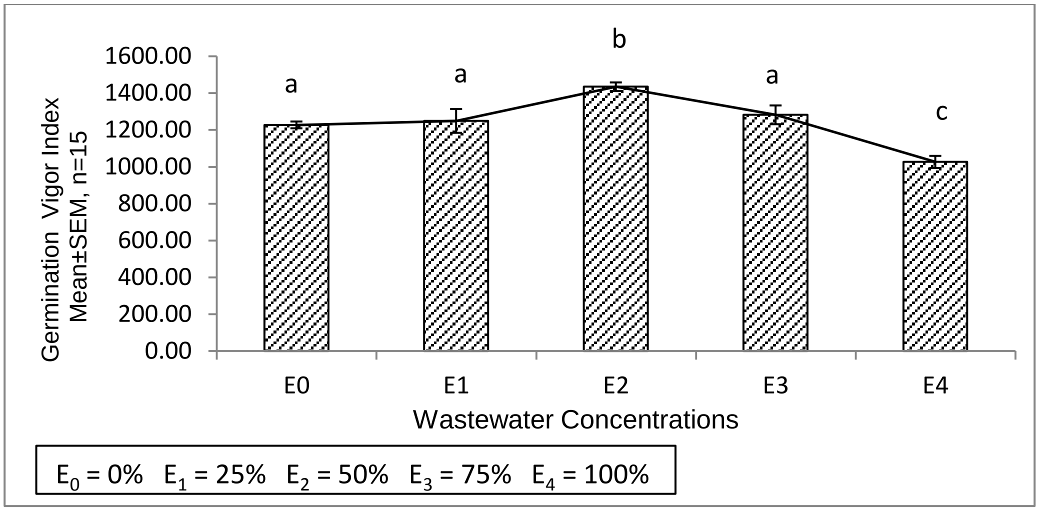

Figure 5.3 Variation in germination vigor index of *Hordeum vulgare* (PL-426) grains at different wastewater concentrations. Data has been analyzed by one way ANOVA, post hoc multiple comparison Tukey's test. Data following same letter are not significantly different by Tukey's test at the 5% level.

5.2.2 Effect on morphological parameters

After 6 days of germination, morphological parameters such as root and shoot length, relative elongation ratio of shoot and root, fresh and dry weight, chlorophyll a and b of *Hordeum vulgare* (PL-426) grains were studied.

5.2.2.1 Root length

Root length of *Hordeum vulgare (*PL-426) grains were affected significantly ($p<0.05$) in response to variation in concentrations of fertilizer industry wastewater. With increase in wastewater up to 50%, there is continuous increase in root length and thereafter it decreased with further increase in wastewater concentration (Fig 5.4). A maximum increase in root length by 31.57% was observed in E_2. The root length in E_4 and E_0 was 4.65 cm and 4.91 cm, respectively showing a significant reduction by 5.29% (E_4) when compared to E_0.

5.2.2.2 Shoot length

Shoot length responded linearly to the increasing concentration of wastewater and ranged from 7.94 cm in E_0 and 8.56 cm in E_2 (Fig 5.5). With shift in wastewater concentration from 50% (E_2) to 100% (E_4), decrease in shoot length was found. Reduction in shoot length by 5.5% was observed in E_4 when compared with E_0. These differences were statistically insignificant ($p>0.05$).

5.2.2.3 Relative elongation of shoot and root

The relative elongation ratio of shoot and root was found to be 101.12% and 109.25%, respectively in E_1. With increase in wastewater concentration from 25% (E_1) to 50% (E_2), an increase in relative elongation ratio of shoot and root was observed with the values of 107.82% and 131.52%, respectively. As concentration of wastewater increases from 50% (E_2) to 100% (E_4), a steady decline in relative elongation ratio of shoot and root of *Hordeum vulgare* (PL-426) grains was observed (Fig 5.6 a and 5.6 b). Minimum value was recorded in E_4 (94.47% and 94.81%, respectively).

5.2.2.4 Fresh weight

Fig 5.7 depicts the variation in fresh weight of the grains with increase in wastewater concentration. Fresh weight of grains was increased up to 5% in E_1 whereas in E_2, it was increased by 26% in comparison to E_0. Beyond 50% (E_2), decrease in fresh weight was observed. Minimum fresh weight was observed in E_4 (0.57g / grain) same as that of E_0. These differences were found to be statistically significant when compared to control ($p<0.05$).

5.2.2.5 Dry weight

A slight increase in dry weight of seedling was noted as we move from E_0 (0.09 g/ grain) to E_2 (0.12 g/ grain). A further increase in wastewater concentration resulted in decline in dry weight of the grain (Fig 5.8). In E_4, the dry weight of the grain was

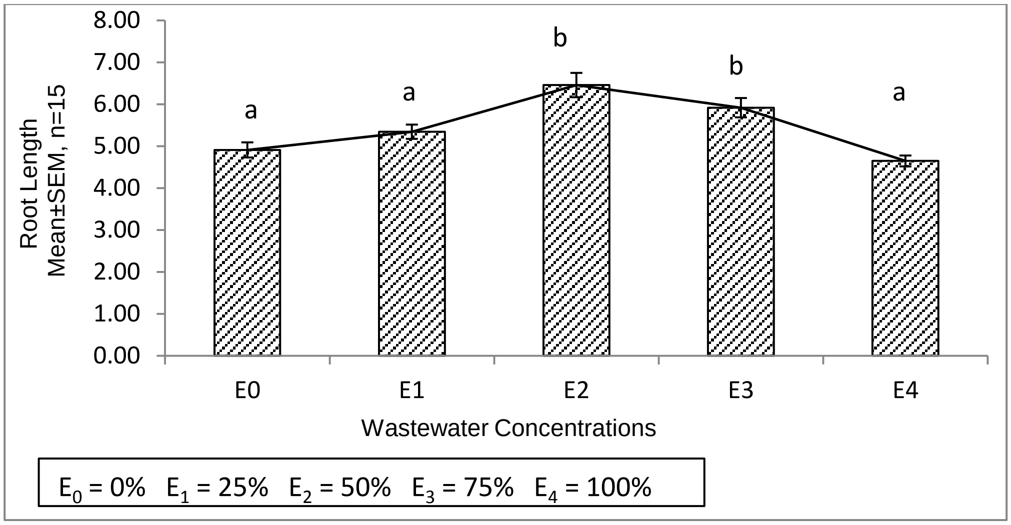

Figure 5.4 Variation in root length of *Hordeum vulgare* (PL-426) grains at different wastewater concentrations. Data has been analyzed by one way ANOVA, post hoc multiple comparison Tukey's test. Data following same letter are not significantly different by Tukey's test at the 5% level.

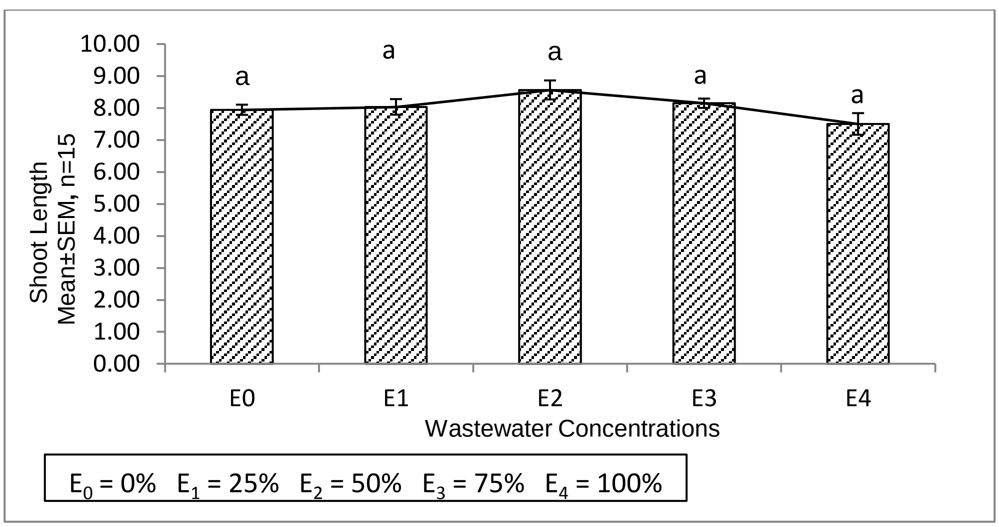

Figure 5.5 Variation in shoot length of *Hordeum vulgare* (PL-426) grains at different concentrations. Data has been analyzed by one way ANOVA, post hoc multiple comparison Tukey's test. Data following same letter are not significantly different by Tukey's test at the 5% level.

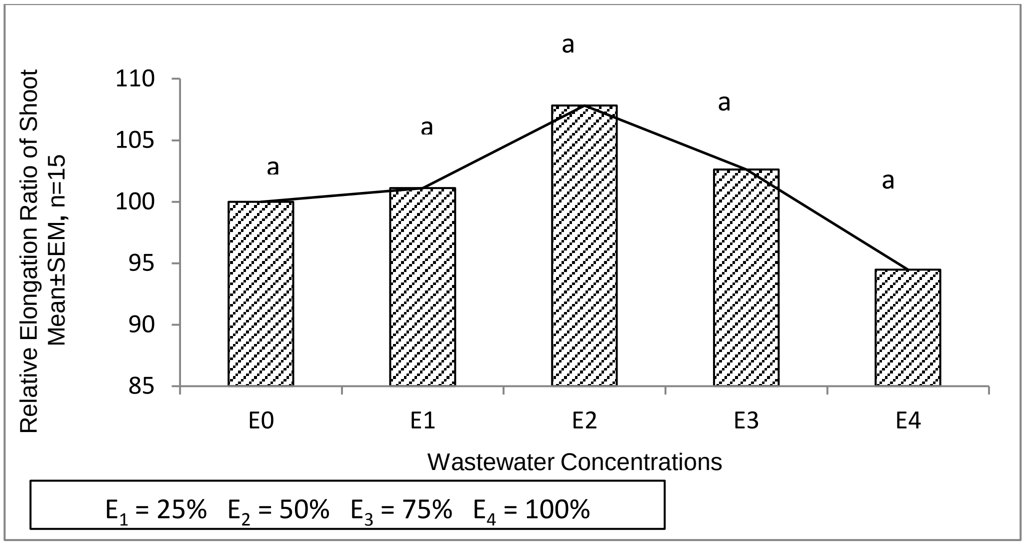

Figure 5.6 a Variation in relative elongation ratio of shoot of *Hordeum vulgare* (PL-426) seeds grains at different wastewater concentrations. Data has been analyzed by one way ANOVA, post hoc multiple comparison Tukey's test. Data following same letter are not significantly different by Tukey's test at the 5% level.

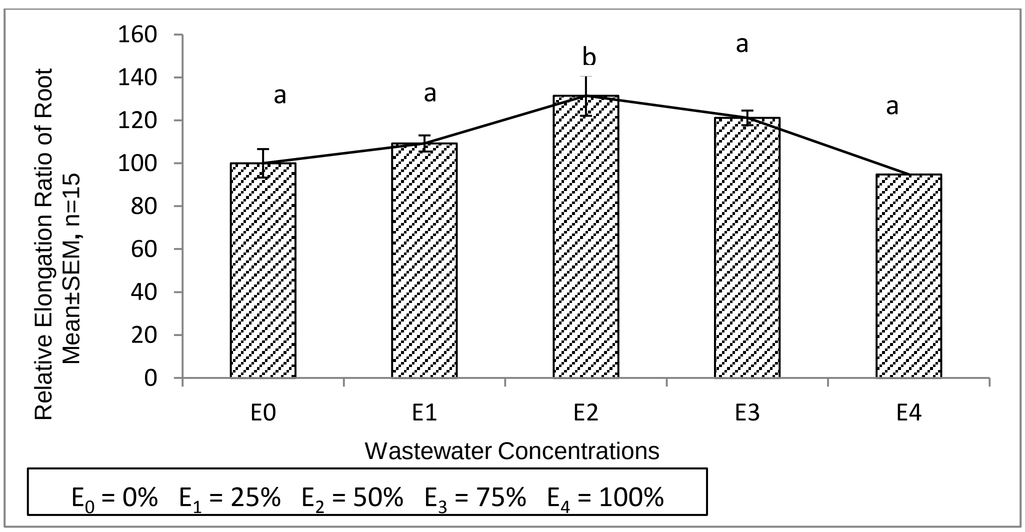

Figure 5.6 b Variation in relative elongation ratio of root of *Hordeum vulgare* (PL-426) grains at different wastewater concentrations. Data has been analyzed by one way ANOVA, post hoc multiple comparison Tukey's test. Data following same letter are not significantly different by Tukey's test at the 5% level.

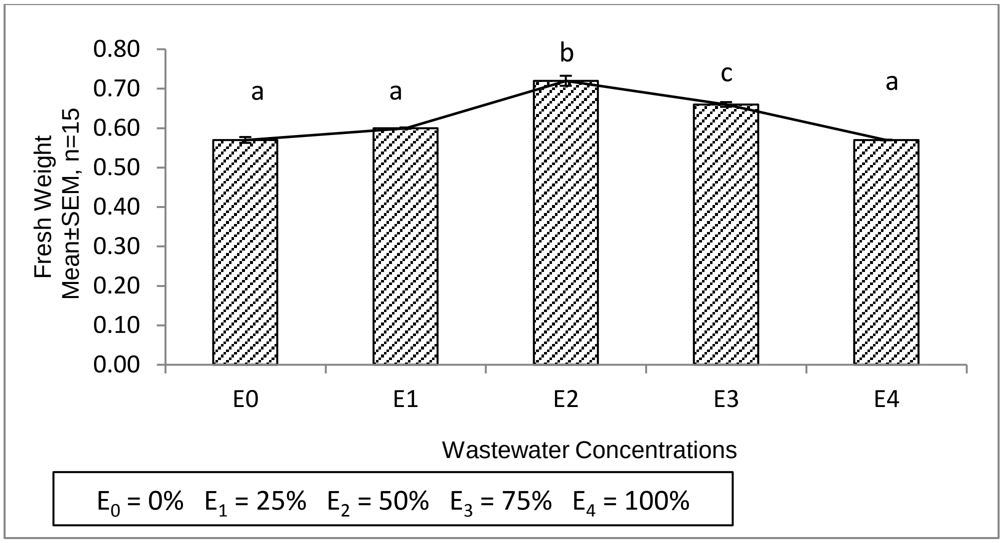

Figure 5.7 Variation in fresh weight of *Hordeum vulgare* (PL-426) grains at different wastewater concentrations. Data has been analyzed by one way ANOVA, post hoc multiple comparison Tukey's test. Data following same letter are not significantly different by Tukey's test at the 5% level.

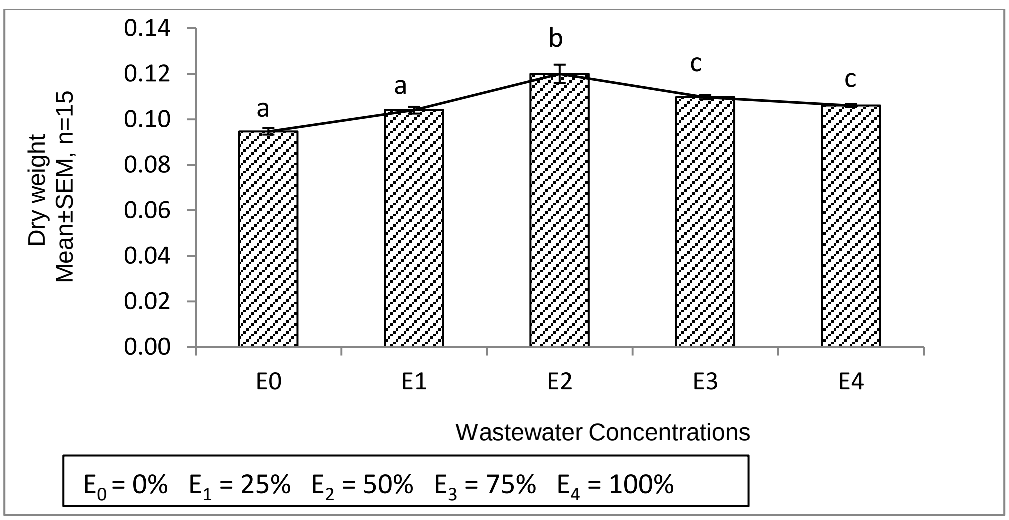

Figure 5.8 Variation in dry weight of *Hordeum vulgare* (PL-426) grains at different wastewater concentrations. Data has been analyzed by one way ANOVA, post hoc multiple comparison Tukey's test. Data following same letter are not significantly different by Tukey's test at the 5% level.

0.11g/ grain. However, minimum dry weight was noted in E_0 (0.09g/ grain). These differences were found to be statistically significant ($p<0.05$).

5.2.2.6 Chlorophyll a and b

Chlorophyll a, b and total chlorophyll content of *Hordeum vulgare* (PL-426) leaves were found to be maximum in E_2 and was around 2.09, 0.56 and 2.64 mg/g fresh weight, respectively whereas in E_0, it was about 2.04, 0.28 and 2.32 mg/g fresh weight, respectively. With increase in wastewater concentrations from 50% (E_2) to 75% (E_3), a decrease in chlorophyll a, b and total chlorophyll contents were observed. However, the decrease was not lesser than the control. When compared to E_0, E_3 showed an increase of 1.47%, 75 % and 10.3%, respectively. A similar increase by about 0.49%, 64% and 7.7%, respectively was observed at E_4 (Fig 5.9 a, 5.9 b and 5.9 c) in comparison to E_0.

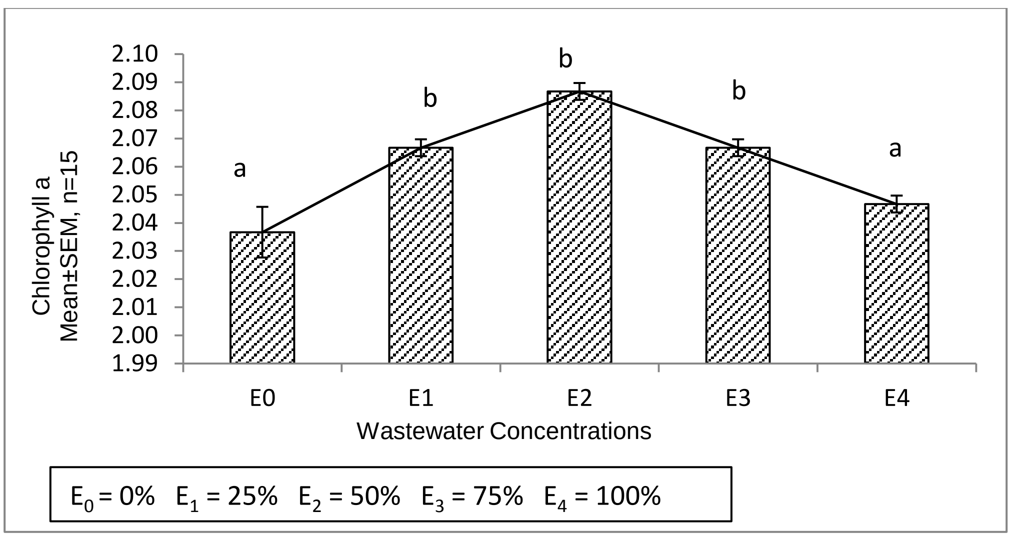

Figure 5.9 a Variation in chlorophyll a content of *Hordeum vulgare* (PL-426) leaves at different wastewater concentrations. Data has been analyzed by one way ANOVA, post hoc multiple comparison Tukey's test. Data following same letter are not significantly different by Tukey's test at the 5% level.

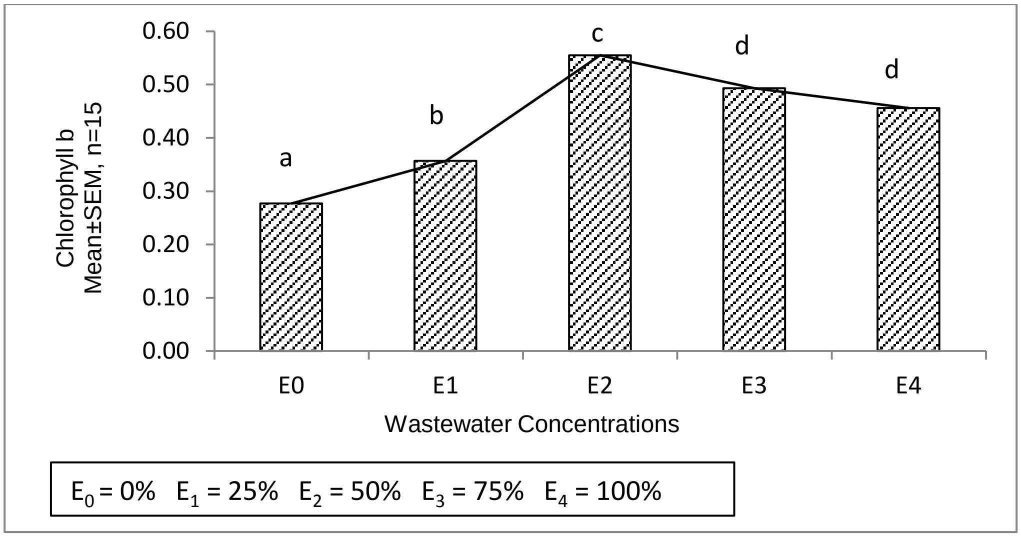

Figure 5.9 b Variation in chlorophyll b content of *Hordeum vulgare* (PL-426) leaves at different wastewater concentrations. Data has been analyzed by one way ANOVA, post hoc multiple comparison Tukey's test. Data following same letter are not significantly different by Tukey's test at the 5% level.

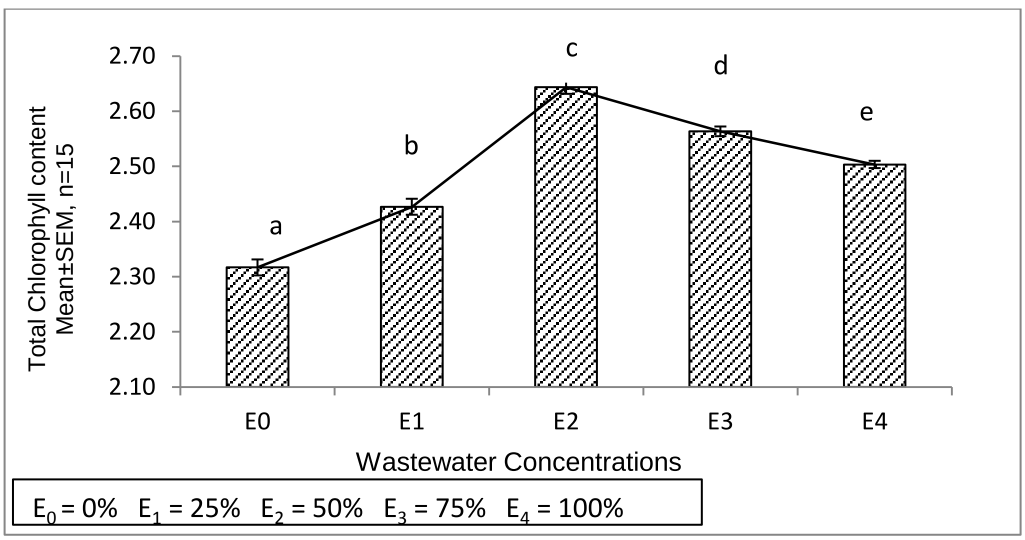

Figure 5.9 c Variation in total chlorophyll content of *Hordeum vulgare* (PL-426) leaves at different wastewater concentrations. Data has been analyzed by one way ANOVA, post hoc multiple comparison Tukey's test. Data following same letter are not significantly different by Tukey's test at the 5% level.

Minimum value of chlorophyll a, b and total chlorophyll contents was noted in E_0. Differences in chlorophyll a, b and total chlorophyll content of *Hordeum vulgare* (PL-426) leaves were found to be statistically significant ($p<0.05$).

5.2.3 Effect on biochemical parameters

The grain was analyzed for biochemical parameters such as α amylase, protease, peroxidase, superoxide dismutase, proteins and carbohydrates at the end of 6 days after the emergence of plumule and radical. It was found that differences in biochemical parameters in different concentrations (E_0, E_1, E_2, E_3 and E_4) were statistically significant ($p<0.05$).

5.2.3.1 α amylase activity

Fig 5.10 depicts the variation in amylase activity of the grains when subjected to different concentrations of wastewater. In E_0, α amylase activity of the grains was observed to be 32.04 ug/min/mg protein. Maximum α amylase activity was recorded in E_4 (34.45 ug/min/mg protein) and a minimum of 23.48 ug/min/mg protein in E_1. An increase by 1.07 times was observed in E_4 when compared with the E_0. α amylase catalyzes the degradation of carbohydrates (Dunn, 1974 and Chang, 1982). Minimum α amylase activity indicates maximum carbohydrates content. E_1 showed minimum amylase activity and maximum carbohydrates content as evident from the Figure 5.14. Differences in α amylase activity of *Hordeum vulgare* (PL-426) grains were statistically significant ($p<0.05$) in comparison to control.

5.2.3.2 Protease activity

Increase in wastewater concentration resulted in a significant ($p<0.05$) decrease in protease activity of *Hordeum vulgare* (PL-426) grains (Fig 5.11). Maximum protease activity of about 0.60 μg/h/mg protein was observed in E_0 and minimum was observed in E_2 (0.37μg/h/mg protein). A significant decrease in protease activity by 0.68 times in E_3 and by 0.66 times in E_4 was observed when compared with the control.

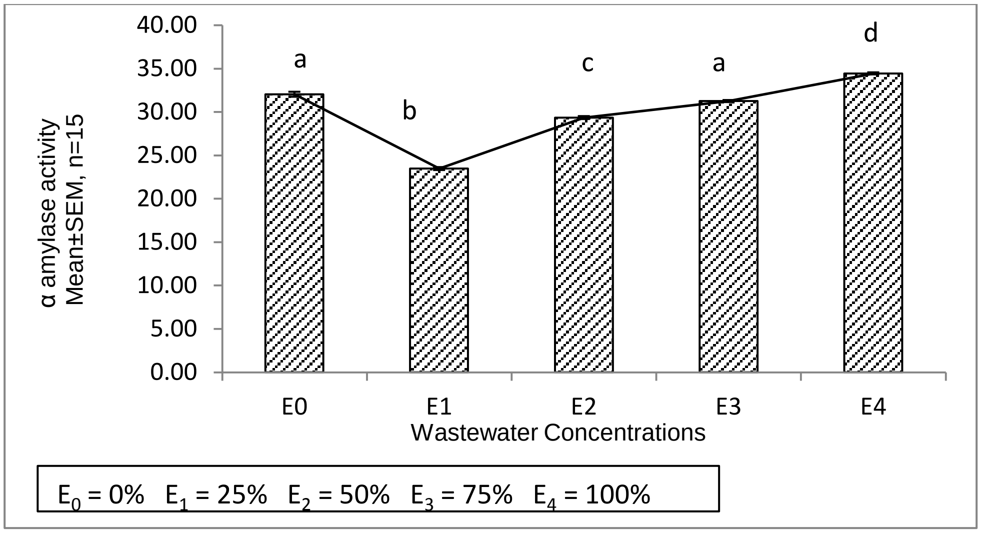

Figure 5.10 Variation in α amylase activity of *Hordeum vulgare* (PL-426) grains at different wastewater concentrations. Data has been analyzed by one way ANOVA, post hoc multiple comparison Tukey's test. Data following same letter are not significantly different by Tukey's test at the 5% level.

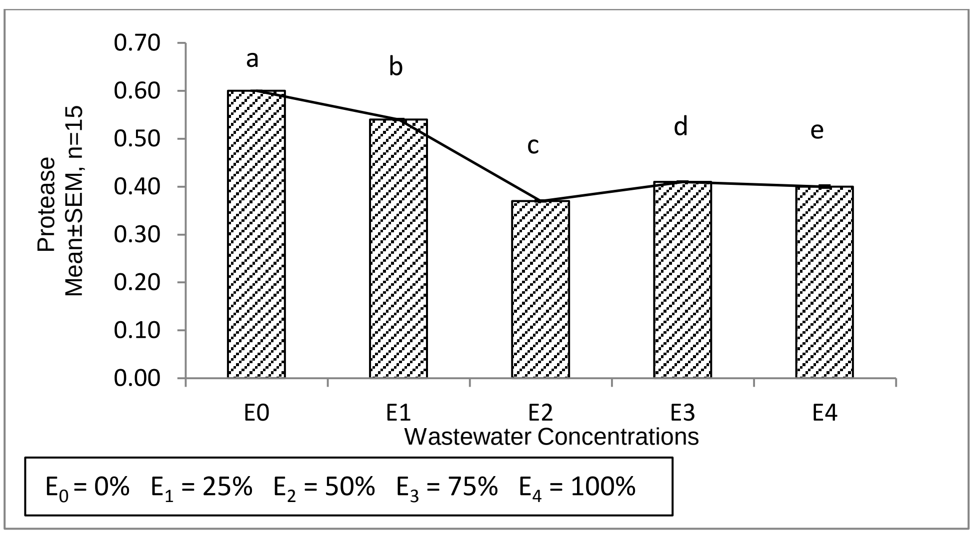

Figure 5.11 Variation in protease activity of *Hordeum vulgare* (PL-426) grains at different wastewater concentrations. Data has been analyzed by one way ANOVA, post hoc multiple comparison Tukey's test. Data following same letter are not significantly different by Tukey's test at the 5% level.

Protease enzyme involves in the hydrolysis of proteins (Berg *et al.*, 2006). Therefore, maximum protease activity in E_0 is an indication of lower protein content in E_0.

5.2.3.3 Antioxidant enzymes activity

Antioxidant enzymes are stress controlling enzymes known to activate under oxidative stress conditions of extreme temperatures and water stress, high light intensity, sulfur dioxide and some pathogens so as to counteract the accumulation of reduced oxygen species (Bowler *et al.*, 1992). The activity of antioxidant enzyme followed a similar trend like all the morphological parameter. Increase in wastewater resulted in significant increase ($p<0.05$) up to 50% (E_2) in comparison to control (E_0). Further, increase in wastewater beyond 50%, resulted in significant decline in antioxidant enzymes of *Hordeum vulgare* (PL-426) grains (Fig. 5.12 a and 5.12 b). In E_2, both peroxidase and superoxide dismutase activities of *Hordeum vulgare* (PL-426) grains was maximum with the value of about 15.79 $min^{-1}g^{-1}$ tissue

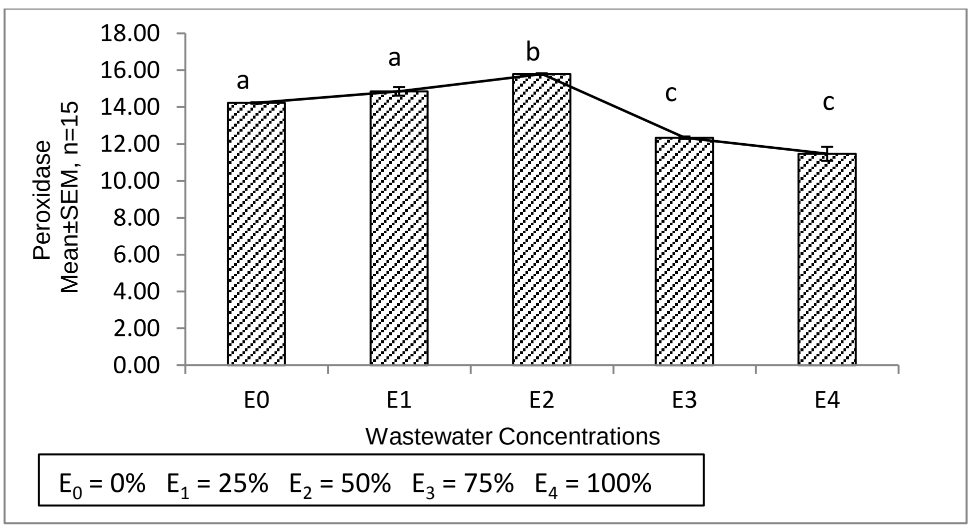

Figure 5.12 a Variation in peroxidase activity of *Hordeum vulgare* (PL-426) grains at different wastewater concentrations. Data has been analyzed by one way ANOVA, post hoc multiple comparison Tukey's test. Data following same letter are not significantly different by Tukey's test at the 5% level.

peroxidase and 61.47$min^{-1}g^{-1}$ fresh weight for superoxide dismutase. It was found that the activity of peroxidase was less than that of E_0 in case of E_3 and E_4 with the value of 12.34$min^{-1}g^{-1}$ tissue and 11.47$min^{-1}g^{-1}$ tissue, respectively. However, superoxide dismutase had higher activity at E_3 (54.5547$min^{-1}g^{-1}$ fresh weight) and E_4 (54.19$min^{-1}g^{-1}$ fresh weight) than that of E_0 (52.48$min^{-1}g^{-1}$ fresh weight).

5.2.3.4 Proteins content

A significant ($p<0.05$) increase in proteins content of *Hordeum vulgare* (PL-426) grains was recorded with increase in wastewater from E_0 (0.71ug/L) to E_2 (1.08ug/L). Maximum value of protein contents was observed in E_2 (1.08ug/L). Further increase in wastewater concentration from 50% (E_2) to 100% (E_4) resulted in slight decline in protein content (Fig 5.13).However, the protein content was still higher as compared to E_0 (0.71ug/L) irrespective of the decline.

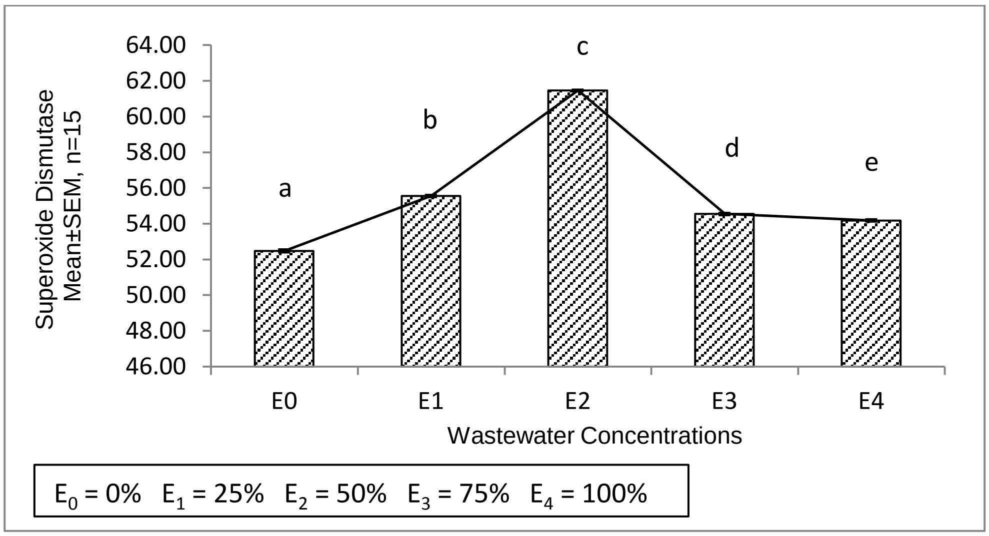

Figure 5.12 b Variation in superoxide dismutase activity of *Hordeum vulgare* (PL-426) grains at different wastewater concentrations. Data has been analyzed by one way ANOVA, post hoc multiple comparison Tukey's test. Data following same letter are not significantly different by Tukey's test at the 95% level.

5.2.3.5 Carbohydrates content

Carbohydrates content of grains of *Hordeum vulgare* (PL-426) increased with increase in wastewater up to 25% (Fig 5.14). Maximum carbohydrates content was observed in E_1 (730.73ug/ml) in comparison to E_0 (719.23ug/ml). Further increase in wastewater resulted in a significant ($p<0.05$) decline in carbohydrates content of *Hordeum vulgare* (PL-426) grains. Minimum carbohydrates content was observed in E_4 (708.07ug/ml).

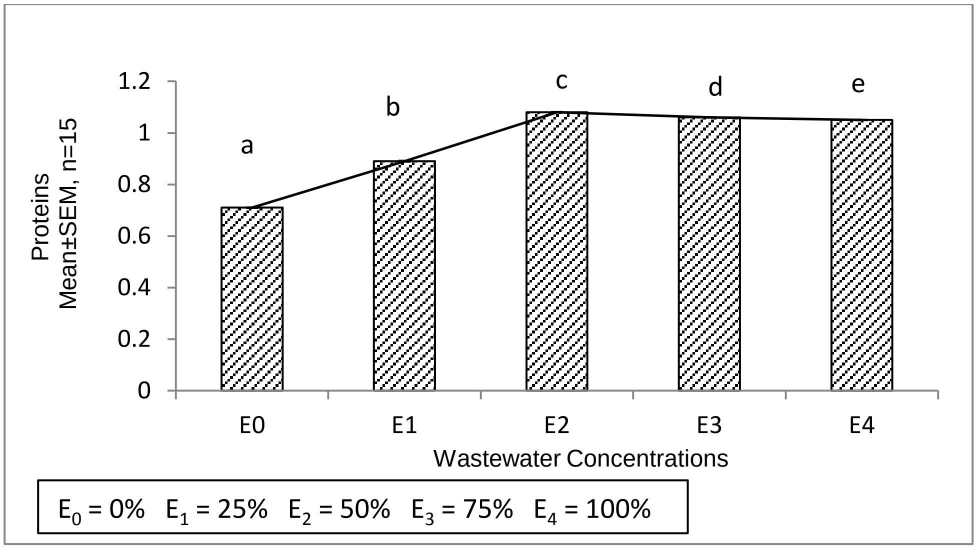

Figure 5.13 Variation in proteins content of *Hordeum vulgare* (PL-426) grains at different wastewater concentrations. Data has been analyzed by one way ANOVA, post hoc multiple comparison Tukey's test. Data following same letter are not significantly different by Tukey's test at the 5% level.

From the above results, it can be concluded that the fertilizer industry wastewater showed best results at 50% of concentration that contained equal ratios of both distilled water as well as fertilizer industry wastewater. By increasing concentration

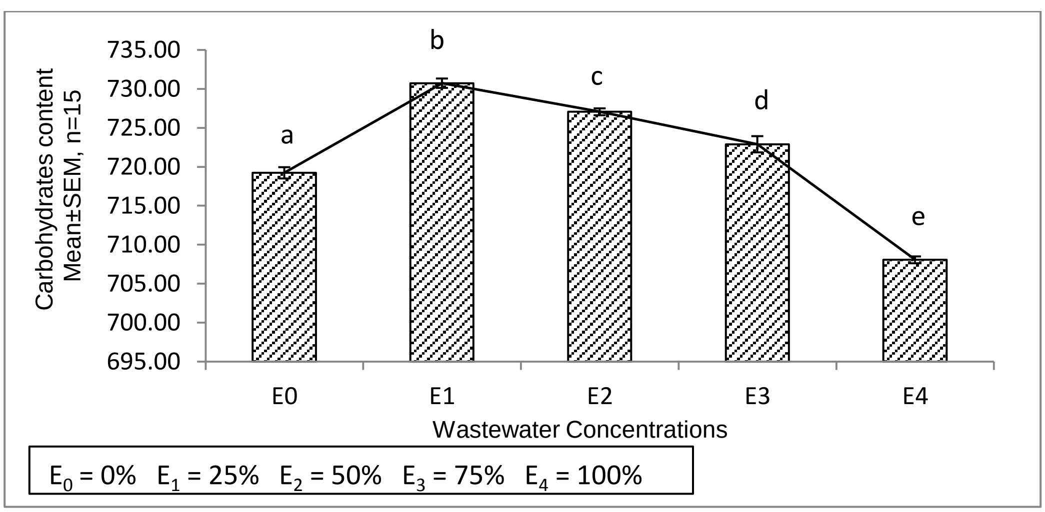

Figure 5.14 Variation in carbohydrates content of *Hordeum vulgare* (PL-426) grains at different wastewater concentrations. Data has been analyzed by one way ANOVA,

post hoc multiple comparison Tukey's test. Data following same letter are not significantly different by Tukey's test at the 5% level.

beyond 50%, significant decrease in different morphological and biochemical parameters of *Hordeum vulgare* (PL-426) grains was observed. Even some of the parameters showed significant decrease in their values of various parameters in comparison to control.

CHAPTER 6

DISCUSSION

This chapter discusses the results obtained from the study conducted to examine the effect of fertilizer industry wastewater on the germination behavior of *Hordeum vulgare* (PL-426) grains.

6.1 Wastewater characterization

The fertilizer industry wastewater was highly acidic. The wastewater has a COD similar to that of a medium strength domestic wastewater. The BOD/COD ratio was around 0.1, indicating poor biodegradability of the wastewater. High COD indicates the presence of elevated concentration of biodegradable organic matter in the wastewater (Kumar and Gopal, 2001). BOD value of 30 mg/L is considered to be appropriate for discharge into inland surface water (IS:2490, 1981). Among the total solids, 50.8% and 37.56% constituted total volatile solids and total dissolved solids, respectively. The TSS constituted 9.24% of total solids. The permissible limits for discharge of industrial wastewater into inland surface water are provided in Table 6.1.

Table 6.1 Prescribed limits for discharge into inland surface water

S.No.	Characteristic	ISI Standards
1	pH	5.5 to 9
2	Biochemical Oxygen Demand (mg/L)	30
3	Chemical Oxygen Demand (mg/L)	250
4	Total Suspended Solids (mg/L)	100
5	Total Dissolved Solids (mg/L)	2100
6	Total Kjeldahl Nitrogen (mg/L)	100
7	Total Ammonical Nitrogen (mg/L)	50
8	Phosphate (mg/L)	5

Source:- IS:2490, 1981

A comparison of the fertilizer industry wastewater characteristics with the permissible limits indicates the requirement of treatment before disposal. Except for ammonical nitrogen, kjeldahl nitrogen and phosphates, rest of the pollutants were above the limits.

6.1.1 Suitability of wastewater for irrigation

pH play a vital role in agriculture as it affects the soil fertility and growth of plant. The pH suitable for agriculture is in the range of 7 - 8. pH greater than standard, affects plant growth. In the present study, pH was highly acidic and it has a definite detrimental effect over plant growth. Distillery wastewater also had same acidic nature like fertilizer wastewater. According to Ale *et al.* (2008), the reason behind low pH might be the presence of higher concentrations of organic acid such as acetic acid. High TDS also has detrimental effects on the plant growth. Rowe and Abdel-Magid (1995) reported that no detrimental effects were observed at TDS value of 500 mg/L. However, TDS between 500 and 1,000 mg/L could affect the sensitive plants and TDS of 1,000- 2,000 mg/L could affect many crops and thus careful management practices were needed. Above 2,000 mg/L, reclaimed water can be used regularly only for tolerant crops on porous soils. High TSS is reported to clog the irrigation system according to Kretschmer *et al.* (2000).

According to Kretschmer *et al.* (2000), only excessive amounts of BOD can cause problems for plant growth. However, low to moderate levels are considered to be beneficial.

6.2 Germination studies of *Hordeum vulgare* (PL-426) grains

Germination is a sign of reproduction and controls dynamics of population and thus is considered as one of the critical test for ensuring crop productivity (Radosevich *et al.*, 1997). The fertilizer industry wastewater caused an insignificant decline in the germination percentage with increasing wastewater concentration from 50% (E_2) to 100% (E_4). Maximum germination percentage of 95.53% was found in distilled water (E_0) and at 50% (E_2) of wastewater concentration. Decrease in germination percentage with increasing wastewater concentration has also been reported by Ramana *et al.* (2002) and Pandey *et al.* (2007) during germination of seed in wheat, pea, lady's finger, cucumber, bottle gourd, onion and chilli with distillery wastewater. According to Ramana *et al.* (2002), maximum germination percentage of 60% was observed in cucumber at 15% of wastewater and around 84% and 80% in onion and chilli at 10% wastewater concentration, respectively. Singh *et al.* (2006) reported the maximum germination percentage of 96% in *Cicer aritenum* at 25% of fertilizer wastewater concentration. The osmotic stress and high COD of wastewater might be the probable reason behind the decreasing germination percentage at higher wastewater concentrations. Because of osmotic stress, water uptake by the grains was reduced which results into the low water contents in germinated embryos that ultimately decrease the germination percentage (Singh *et al.*, 2006). High COD of wastewater resulted in increased ionic strength due to which the transfer of water to the grain slowed down. As a result, the grain did not get sufficient water needed for its germination, so reduction in germination percentage was seen.

Similar to the other parameters, relative elongation ratio of root and shoot increased up to 50% (E_2) of fertilizer industry wastewater after which a steady decline was observed. The results obtained in the present study are in accordance with the results obtained by Begum *et al.* (2010) while studying the effect of natural gas fertilizer factory wastewater on seeds of mustard, amaranth stem, radish and red amaranth. Maximum shoot elongation ratio of 151.60% and 118.67% was observed in seeds of radish and red amaranth, respectively at 100% wastewater. Seeds of mustard and amaranth showed respective increase in shoot elongation ratio (108.25% and 123.23%) at 75% and 25% wastewater, respectively. Root elongation ratio also showed similar results as shoot elongation ratio in the study conducted by Begum *et al*. (2010).

Germination index increased up to 50% (E_2) of fertilizer industry wastewater and later it started declining. Maximum germination index of 131.53% was observed at 50% of wastewater. However, at 100%, the germination index was observed to be lesser than that of control. In addition to the above mentioned reasons, the reduction in germination index with increasing wastewater concentration might be due to the presence of recalcitrant compounds. Additionally, the acidic pH also could have impaired the germination index. These results are in agreement with earlier studies by Singh *et al.* (2009), while studying the impact of monosodium glutamate industrial wastewater on growth of Chinese cabbage and maize. Maximum germination index was observed at 0.2% wastewater. Malaviya and Sharma reported maximum germination index of 103.2% in *Brassica napus* L. at 20% concentration of distillery wastewater.

Like other parameters, the germination vigor index also showed similar trend that attaining maximum at 50% (E_2) of fertilizer industry wastewater. Sufficient amount of nutrients at 50% wastewater could be the possible reason behind the increase. However, these nutrients might not able to support germination at higher concentrations. Contradicting the results of the present study, Kanan and Upreti (2008) observed maximum value of vigor index (1270%) in control while studying effects of treated and untreated distillery effluents on the germination and growth of mung bean seeds.

Root and shoot length of *Hordeum vulgare* (PL-426) showed maximum value at 50% fertilizer industry wastewater than control (E_0). A further increase in wastewater percentage (E_3 and E_4) resulted in significant decrease in root and shoot length. The high TDS value of wastewater might be the probable reason behind decrease in grain growth. At high TDS, the water moves out of the grain making the nutrients unavailable for its proper growth. Similar results have been reported by Nath *et al.* (2007) while studying the combinatorial effects of distillery and sugar factory wastewater on barley. They reported maximum root and shoot length of 5.53 and 9.53 cm, respectively at 50% of wastewater concentration. On the other end, Nagajyothi *et al.* (2009) observed maximum root and shoot length of 4.8 and 26.8 cm/seedling, respectively at 25% of wastewater concentration while growing green gram in biomass power plant wastewater.

In the present study, both fresh and dry weight of *Hordeum vulgare* (PL-426) increased with increase in fertilizer industry wastewater concentration up to 50%. Beyond 50% (E_2), significant decline in fresh and dry weight was seen. Thus increase in wastewater caused decrease in fresh and dry weight. The results have been in agreement with the Dhanam (2009) while observing effect of dairy wastewater on paddy. Increase in fresh and dry weight at lower wastewater concentration might be due to the availability of desired levels of nutrient in diluted form but at high wastewater concentrations, they might be toxic (Ale *et al.*, 2008).

Photosynthetic pigments such as total chlorophyll, chlorophyll a and b increased significantly up to 50% fertilizer industry wastewater. At higher wastewater concentrations decrease in the pigments was observed. Similar observation was also reported by Nath *et al.* (2007), while studying the combinatorial effects of distillery and sugar factory wastewater on barley. Nath *et al.* (2007) reported a total chlorophyll of 2.721 mg/g fresh weight at 50% wastewater concentration while Singh *et al.* (2006) reported maximum values of chlorophyll a (11.41 mg/g Fresh Weight) and chlorophyll b (12.65 mg/g Fresh Weight) at 25% concentration of wastewater. According to Singh *et al.* (2006), the probable reason behind the chlorophyll inhibition might be due to the induced inhibition of the electron transport system in PS-II. A significant decrease in chlorophyll content at higher concentrations of wastewater may be because of the inhibitory effects of toxicants on chlorophyll synthesis in plant.

Carbohydrates are essential to produce energy required for various metabolic processes and also for the process of intensive differentiation and growth under steady state conditions. When plants are exposed to environmental stress, disturbance in energy forming molecules takes place. As a result, alteration in the protein and carbohydrates metabolites occurs (Easwari and Lalitha, 1995). In the present study, the carbohydrate was observed to increase from control to 25% of fertilizer industry wastewater beyond which carbohydrates contents started decreasing. Decrease in carbohydrate at higher concentration may be due to increase in α amylase activity. Similar conclusions were also given by Dhanam (2009) while studying dairy wastewater effect on paddy and reported a value of 8.264 mg/g fresh weight at 25% of wastewater concentration. However, proteins content was found to be maximum at 50% (E_2) of wastewater concentration. Increase in fertilizer industry wastewater from 50% (E_2) to 100% (E_4) caused decrease in protein content of *Hordeum vulgare* (PL-426). Similar findings have been given by Sukanya and Meli (2003) while working on wheat and distillery wastewater. Contradicting to the above results, Paliwal *et al.* (1998) observed maximum protein content in *Hardwickia binata* at 75% sewage water.

Plants are consistently exposed to several forms of stress (drought, heat, chilling and pollutants) during normal conditions of growth and development. These stress conditions leads to the generation of reactive oxygen species (ROS) such as superoxide radicals, singlet oxygen, H_2O_2 and hydroxyl radicals (Scandalios, 1993; Allen, 1995; Anderson *et al.*, 1995 and Rao *et al.*, 1996). ROS damage the cell structures (DNA, proteins, membrane lipids) thereby disrupting the homeostasis of organism (Shaaltiel and Gressel, 1986 and Scandalios, 1993). Plants antioxidant defense machinery such as superoxide dismutase and peroxidase protects them against the damage caused by oxidative stress through scavenging of ROS. In the present study, both peroxidase and superoxide dismutase enzymes showed maximum activity at 50% of fertilizer industry wastewater. Conversely, further increase in wastewater caused significant decrease in their activities. Nath *et al.* (2009) also observed an increase in peroxidase activity of 34.56 ΔO.D./g in radish up to 50% concentration while working with tannery wastewater. Singh *et al.* (2003) recorded maximum peroxidase activity when plants are grown with NPK+FYM, (Nitrogen Phosphorus Potassium+Farm Yard Manure) biomethanated and raw spent wash which were more than that of control. Baskaran *et al.* (2009) observed lethal effect of sugar mill wastewater from 25% concentration onwards.

α amylase and protease are hydrolyzing enzymes known to carry out the hydrolysis of carbohydrates and proteins, respectively. α amylase play an important role in starch hydrolysis and release of energy (Dunn, 1974 and Chang, 1982). On studying the effect of fertilizer industry wastewater on hydrolyzing enzymes such as α- amylase and protease, it was found that α-amylase activity was higher at 100% of fertilizer industry wastewater and protease activity was found to be maximum at 0% of wastewater. Nath *et al.* (2007) reported maximum α-amylase activity in control in case of wheat, garden pea, black gram and mustard while in barley, maximum α-amylase activity was observed at 50% of distillery and sugar factory mixed wastewater. Protease activity was maximum in control and it started decreasing with increasing wastewater concentration. Antolin *et al.* (2005) observations contradicted the results of present study, where an increase in protease activity in barley was observed when treated with sewage sludge.

The activity of amylase was negatively correlated (p=-0.87) to carbohydrates. Similar strong negative correlation (p=-0.987) also exists between protease activity and proteins content. The possible reason behind this would be that both amylase and protease are hydrolyzing enzymes that hydrolyze carbohydrates and proteins, respectively. So, with increase in amylase and protease activities, corresponding decrease in carbohydrates and protein contents was observed. The data was further extended to regression analysis. The R^2 values for amylase-carbohydrates and protease-protein came out to be 0.76 and 0.94, respectively. Since, the p value for amylase-carbohydrates is 0.03 and for protease-protein are 0.0009, which is less than 0.05. It could be concluded that the regression parameters are statistically significant at significance level 0.05.

The study clearly indicated that up to certain dilution level, fertilizer wastewater could be an additional source of water. Good growth was seen at 50% of fertilizer industry wastewater in most of the parameters (germination percentage, , germination Index, root length, shoot length, relative elongation ratio of shoot, relative elongation ratio of root, fresh weight, dry weight, chlorophyll a and b, peroxidase, superoxide dismutase and proteins) but in case of carbohydrates, maximum value was observed at 25% of wastewater concentration. Hydrolyzing enzymes such as α amylase and protease showed different pattern. Activity of α amylase was found to be maximum at 100% wastewater whereas protease enzymes showed maximum activity in control. Values of some of the parameters like dry weight, chlorophyll a, b and total chlorophyll, superoxide dismutase and proteins were still more when compared with the control.

CHAPTER 7

SUMMARY AND CONCLUSION

7.1 Summary

In the present study, the effect of fertilizer industry wastewater, on germination behaviour of *Hordeum vulgare* (PL-426) grains was examined. Experimental work includes (1) collection and characterization of fertilizer industry wastewater for various physico-chemical parameters and (2) to study its effect on germination by assessing the various morphological and biochemical parameters of *Hordeum vulgare* (PL-426) grains.

The barley grains were germinated in petridishes designated as E_0 (control), E_1 (25% wastewater), E_2 (50% wastewater), E_3 (75% wastewater) and E_4 (100% wastewater), according to the different wastewater concentration. After 6 days of germination, the grains growth was assessed based on morphological (germination percentage, germination index, germination vigor index, root and shoot length, relative elongation ratio of shoot and root, fresh and dry weight, chlorophyll a, b and total chlorophyll content) and biochemical (α amylase and protease, peroxidase, superoxide dismutase, proteins and carbohydrates) parameters.

Table 7.1 summarizes the results obtained during the present study. From the table, it is evident that most of the growth parameters showed maximum value at 50% of wastewater except for some parameters such as carbohydrates, α amylase and protease. In 50% wastewater, the root and shoot length of *Hordeum vulgare* (PL-426) grains had maximum increase by 31.5% and 7.8%, respectively in comparison to control. Germination index was found to increase by 31.5% in 50% wastewater. Peroxidase enzyme showed about 13.2% and 19.3% decrease at 75% and 100%, respectively as compared to control. Some parameters (dry weight, chlorophyll a, b and total chlorophyll content, superoxide dismutase and proteins) showed more growth even at 100% of wastewater when compared to that of control. Total chlorophyll showed 7.75% increase whereas chlorophyll b (64.2%) showed more percent increase than chlorophyll a (0.49%) at 100% of wastewater in comparison to control. Antioxidant enzyme such as superoxide dismutase showed around 3.26% increase as compared to control at 100% wastewater. With increase in dilution levels from 50% to 100%, no further increase in growth was seen. Conversely, α amylase and protease showed maximum values at 100% and 0% of wastewater, respectively. At 50%, α amylase activity of *Hordeum vulgare* (PL-426) grains showed around 8.3% decrease whereas protease enzyme showed 38.3% decrease with respect to control.

From, the present study it is clearly evident that at 50% dilution level containing equal volumes of distilled water and fertilizer industry wastewater is appropriate for the germination of *Hordeum vulgare* (PL-426) grain.

7.2 Conclusions

The major findings of the present study concluded that

- The fertilizer industry wastewater showed the presence of high concentration of solids exceeding the wastewater discharge standards.
- The organic fraction of the wastewater showed a similar strength as that of medium strength domestic wastewater.
- The growth was higher at 50% wastewater compared to control indicating that the wastewater enhanced the growth when equally diluted with distilled water. However at higher concentrations it had an inhibitory effect on the germination and growth.
- The differential response of the grains at higher wastewater concentrations might be due inhibitory effects of the pollutants at higher concentrations.
- The decrease in enzyme activity indicated that the plant was stressed at higher wastewater concentrations.

Table 7.1 Effect of different concentrations of fertilizer industry wastewater on germination rate, morphological and biochemical parameters of *Hordeum vulgare* (PL-426) grains.

Parameters studied		Wastewater Concentrations								
		E_0	E_1		E_2		E_3		E_4	
		mean± S.D.	mean± S.D.	Percent change(%)	mean± S.D.	Percent change (%)	mean± S.D.	Percent change (%)	mean± S.D.	Percent change (%)
Germination Rate	Germination percentage (%)	95.53± 3.87	93.33± 6.65	2.3% decrease	95.53± 3.87	0	91.10± 3.81	4.6% decrease	84.47± 3.87	11.5% decrease
	Germination Index (%)	100.00± 0.00	106.70± 13.00	6.7% increase	131.53± 6.61	31.5% increase	115.42± 13.98	15.4% increase	83.69± 2.75	16.3% decrease
	Germination Vigor Index	1227.32± 31.91	1248.92± 111.95	0.017% increase	1434.35± 40.76	0.168% increase	1282.03± 88.12	0.044% increase	1026.29± 56.99	0.163% decrease
Morphological parameters	Root Length (cm)	4.91± 0.30	5.34± 0.30	8.7% increase	6.46± 0.50	31.5% increase	5.92± 0.40	20.5% increase	4.65± 0.22	5.2% decrease
	Shoot Length (cm)	7.94± 0.15	8.03± 0.24	1.1% increase	8.56± 0.30	7.8% increase	8.15± 0.15	2.6% increase	7.50± 0.34	5.5% decrease

	Relative elongation ratio of shoot (%)	100± 0.00	101.12± 1.26	1.12% increase	107.82± 3.92	7.8% increase	102.60± 3.73	2.6% increase	94.47± 5.43	5.5% decrease
	Relative elongation ratio of root (%)	100± 0.00	109.25± 11.5	9.25% increase	131.52± 6.52	31.52% increase	121.17± 16.25	21.17% increase	94.81± 5.90	5.19% decrease
	Fresh weight (g/ grain)	0.57± 0.013	0.60± 0.003	5.2% increase	0.72± 0.022	26.3% increase	0.66± 0.010	15.7% increase	0.57± 0.001	0
	Dry weight (g/ grain)	0.09± 0.002	0.10± 0.003	11.1% increase	0.12± 0.007	33.3% increase	0.11± 0.001	22.2% increase	0.11± 0.001	22.2% increase
	Chlorophyll a (mg/g Fresh Weight)	2.04± 0.015	2.07± 0.006	1.4% increase	2.09± 0.006	2.4% increase	2.07± 0.006	1.4% increase	2.05± 0.006	0.49% increase
	Chlorophyll b (mg/g Fresh Weight)	0.28± 0.01	0.36± 0.02	28.5% increase	0.56± 0.01	100% increase	0.49± 0.01	75% increase	0.46± 0.01	64.2% increase
	Total chlorophyll content (mg/g Fresh	2.32±0.02	2.43±0.02	4.74% increase	2.64±0.02	13.79% increase	2.56±0.01	10.34% increase	2.50±0.01	7.75% increase

	Weight)									
Biochemical parameters	α amylase (ug/min/mg protein)	32.04± 0.50	23.48± 0.29	26.7% decrease	29.35± 0.29	8.3% decrease	31.26± 0.21	2.4% decrease	34.45± 0.21	7.5% increase
	Protease (µg/h/mg protein)	0.60± 0.001	0.54± 0.002	10% decrease	0.37± 0.002	38.3% decrease	0.41± 0.002	31.6% decrease	0.40± 0.005	33.3% decrease
	Peroxidase ($min^{-1}g^{-1}$ tissue)	14.23± 0.06	14.86± 0.39	4.4% increase	15.79± 0.09	10.9% increase	12.34± 0.13	13.2% decrease	11.47± 0.65	19.3% decrease
	Superoxide dismutase ($min^{-1}g^{-1}$ Fresh Weight)	52.48± 0.16	55.55± 0.11	5.8% increase	61.47± 0.09	17.1% increase	54.55± 0.13	3.9% increase	54.19± 0.13	3.2% increase
	Proteins (µg/L)	0.71± 0.002	0.89± 0.003	25.3% increase	1.08± 0.002	52.1% increase	1.06± 0.001	49.2% increase	1.05± 0.001	47.8% increase
	Carbohydrate (µg/ml)	719.23± 1.25	730.73± 1.04	1.6% increase	727.07± 0.76	1.1% increase	722.90± 1.80	0.51% increase	708.07± 0.76	1.5% decrease

Overall, it can be concluded that the use of fertilizer industry wastewater with proper dilution provides a probable solution for management of wastewater. The application of wastewater to agriculture provides an eco friendly method of disposal. However, further study is required in order to explore the effect of wastewater on soil before being used for field application (irrigation).

7.3 Future perspectives

- The reproducibility of the present study should be examined.
- To find out the contributing factor responsible for decreasing growth rate beyond 50%.
- Extension of the present study to pot culturing.
- Similar studies can be extended for different wastewaters such as sewage, distillery, tannery etc and its effect on the germination and crop performance can be studied.
- Field studies on the effect of wastewater on crops can be done.
- Change in soil characteristics after wastewater application can be studied.
- Comparative study on effect of wastewater as such and in combination with various organic and inorganic fertilizers on crops can be initiated.

REFERENCES

Abdul-Baki, A.A. and Anderson, J.D. (1973). Vigor determination soyabean seed by multiple criteria. *Crop Science* **13**: 630-633.

Adoki, A. and Orugbani, T. (2007). Influence of nitrogenous fertilizer plant effluents on growth of selected farm crops in soils polluted with crude petroleum hydrocarbons. *African Journal of Agricultural Research* **2**: 569-573.

Ahmad, I., Ali, S. and Jan, M.R. (2000). Physico-chemical analysis of fertilizer industry effluent and its effects on crop plants. *Pakistan Journal of Scientific and Industrial Research* **3**: 83-89.

Ale, R., Jha, P.K. and Belbase, N. (2008). Effects of distillery effluent on some agricultural crops: A case of environmental injustice to local farmers in Khajura VDC, Banke. *Scientific World* **6**: 68-75.

Al-Jamal, M.S., Sammis, T.W., Mexal, J.G, Picchioni, G.A. and Zachritz W.H. (2002). A growth-irrigation scheduling model for wastewater use in forest production. *Agricultural Water Management* **56**: 57-59.

Al-Lahham, R., El-Assi, N.M. and Fayyad, M. (2003). Impact of treated wastewater irrigation on quality attributes and contamination of tomato fruit. *Agricultural Water Management* **61**: 51-62.

Allen, R.D. (1995). Dissection of oxidative stress tolerance using transgenic plants. *Plant Physiology* **107**: 1049-1054.

Anderson, M.D., Prasad, T.K. and Stewart, C.R. (1995). Changes in isozyme profiles of catalase, peroxidase and glutathione reductase during acclimation to chilling in mesocotyls of maize seedlings. *Plant Physiology* **109**: 1247-1257.

Antolin, M.C., Pascual, I., Garcia, C., Polo, A. and Sanchez-diaz, M. (2005). Growth, yield and solute content of barley in soils treated with sewage sludge under semiarid Mediterranean conditions. *Field Crop Research* **94**:224-237.

APHA (2005). Standard method for the examination of water and wastewater. 21st Edition American Public Health Association, AWWA, WPC.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiology* **24**: 1-15.

Aziz, O., Inam, A. and Samiullah (1999). Utilization of Petrochemical industry wastewater for agriculture. *Water, Air and Soil Pollution* **115**: 321-335.

Baghel, R.S. (2008).Toxicity of distillery effluent on seed germination, seedling growth and metabolism in *Pisum sativum*. *Research in Environment and Life Sciences* **1**: 29-32.

Basha, S.M.M. and Beevers, L. (1975). The development of proteolytic activity and protein degradation during the germination of *Pisum sativum* L. *Planta* **124**: 77-87.

Baskaran, L., Sundaramoorthy, P., Chidambaram, A.L.A. and Ganesh, K.S. (2009). Growth and physiological activity of green gram (*Vigna radiata* L.) under effluent stress. *Botany Research International* **2**: 107-114.

Bazai, Z.A. and Achakzai, A.K.K. (2006). Effect of wastewater from Quetta city on the germination and seedling growth of Lettuce (*Lactuca sativa L*.). *Journal of Applied Sciences* **6**: 380-382.

Begum, S.A., Alam, M.J., Rahman, S.S. and Rahman, M.M. (2010). Effect of industrial effluents on the germination and seedling growth of three leafy vegetables. *Bangladesh Journal of Scientific and Industrial Research* **45**: 101-104.

Behera, B. and Reddy, V.R. (2002). Environment and accountability: Impact of industrial pollution on rural communities. *Economic and Political Weekly* **37**: 257-265.

Berg, J.M., Tymoczko, J.L. and Stryer, L. (2006). *Biochemistry*, pp. 243. W.H. Freeman and Company, New York.

Bharagava, R.N., Chandra, R. and Rai, V. (2008). Phytoextraction of trace elements and physiological changes in Indian mustard plants (*Brassica* *nigra L.)* grown in post methanated distiller effluent (PMDE) irrigated soil. *Bioresource Technology* **99**: 8316-8324.

Bhatti, M. and Singh, G. (2003). Growth and mineral accumulation in *Eucalyptus camaldulensis* seedlings irrigated with mixed industrial effluents. *Bioresource Technology* **88**: 221-228.

Bowler, C., Montagu, M.V. and Inze, D. (1992). Superoxide dismutase and stress tolerance. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**: 83-116.

Brito, J.C., Velica, A., Guerrero, C., Beltrao, J. and Faleiro, M.L. (1997). Reuse of municipal sludges and industrial wastes as organic fertilizers in tomato. *Acta Horticulturae* **449**:707-714.

Bureau of Indian Standards (1989). Method of sampling and test (physical and chemical) for water and wastewater. IS : 3025 (Part 34).

Burun, B., Tuna, A.L., Yokas, I. and Ege, H. (2006). Some soil properties of lime stabilized urban wastewater and effects on barley's yield and mineral matter contents. *Journal of Agronomy* **5**: 37-44.

Carter, C.T., Grieve, C.M., Poss, J.A. and Suarez, D.L. (2005). Production and ion uptake of *Celosia argentea* irrigated with saline wastewater. *Scientia Horticulturae* **106**: 381-394.

Chandraju, S., Siddappa and Chidankumar, C.S. (2011). Studies on the germination and growth of cotton and groundnut seeds irrigated by distillery spentwash. *Current Botany* **2**: 38-42.

Chang, C.W. (1982). Enzymic degradation of starch in cotton leaves. *Phytochemistry* **21**: 1263-1269.

Chidankumar, C.S., Chandraju, S. and Nagendraswamy, R. (2009). Impact of distillery spentwash on the yields of top vegetables (Creepers). *World Applied Sciences Journal* **6**: 1270-1273.

[CPCB] Central Pollution Control Board. (2009). Status of water supply, wastewater generation and treatment in class-I cities and class-II towns of

India. <http://www.cpcb.nic.in/upload/NewItems/NewItem_153_Foreword.pdf>. Accessed 2012 Jan 3.

Dane, F., Ekici, N. and Aktas, Y.K. (2006). The effect of wastewater on root growth and mitosis in onion (*Allium cepa*) root apical meristem. *Asian Journal of Plant Science* **5**: 331-334.

Dhanam, S. (2009). Effect of dairy effluent on seed germination, seedling growth and biochemical parameter in paddy. *Botany Research International* **2**: 61-63.

Doke, K.M., Khan, E.M., Rapolu, J. and Shaikh, A. (2011). Physico-chemical analysis of sugar industry effluent and its effect on seed germination of *Vigna angularis*, *Vigna cylindrical* and *Sorghum cernum*. *Annals of Environmental Science* **5**: 7-11.

Dunn, G. (1974). A model for starch breakdown in higher plants. *Phytochemistry* **13**: 1341-1346.

Easwari, K. and Lalitha, K. sub-cellular distribution of selenium during uptake and influence on mitochondrial oxidation in *Vigna radiata* L. *Biological Trace element Research* **48**: 141-160.

[EOE] Encyclopedia of Earth (2012). <http://www.eoearth.org/article/Water_pollution?topic=58075>. Accessed 2012 May 5.

[ENVIS] Environment Information System. (1994).

<http://cpcbenvis.nic.in/newsletter/agro-dec-1994/dec941.htm>. Accessed 2012 Jan 3.

[EPR] The Environment Protection Rules. (1986).

<http://www.moef.nic.in/legis/env/env4.html>. Accessed 2012 Jan 27.

Gaikar, R.B., Uphade, B.K., Gadhave, A.G. and Kuchekar, S.R. (2010). Effect of dairy effluent on seed germination and early seedling growth of soybeans. *Rasayan Journal of Chemistry* **3**: 137-139.

Garg, V.K. and Kaushik, P. (2008). Influence of textile mill wastewater irrigation on the growth of sorghum cultivars. *Applied Ecology and Environmental Research* **6**: 1-12.

Giri, A.N. (2008). Effect of paper and pulp mill effluent on germination, growth, biochemical parameters and bioaccumulation of heavy metal in *Pisum sativum. Research in Environment and Life Sciences* **1**: 61-64.

Hiscox, J.D. and Israelstam, G.F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany* **57**: 1332-1334.

Hussain, F., Khattak, S.K. and Haq, I.U. (1982). Effect of effluents from Adamjee paper and board mills (Nowshera) on the germination and growth of some cultivated plants. *Pakistan Journal of Agricultural Research* **3**: 182-194.

Hussain, F., Malik, S.A., Mohammad, A., Bashir, N., Younis, U., Hassan, M. and Mahmood, S. (2010). Effect of tannery effluents on seed germination and growth of two sunflower cultivars. *African Journal of Biotechnology* **9**: 5113-5120.

IS:2490 (Part I) (1981). Tolerance limits for industrial effluents

<http://www.worldenviro.com/effstd.htm>. Accessed 2012 Mar 27.

Juwarkar, A. and Dutta, S.A. (1989). Impact of distillery effluent application to land on soil microflora. *Environmental Monitoring and Assessment* **15**: 201-210.

Kamalakar, J.A., Sharma, D. and Melkania, U. (1991). Effect of paper and pulp factory effluents on the growth and development of maize and sunflower. *International Journal of Environmental Studies* **39**: 55-63.

Kannan, A. and Upreti, R.K. (2008). Influence of distillery effluent on germination and growth of mung bean (*Vigna radiata*) seeds. *Journal of Hazardous Materials* **153**: 609-615.

Kaushik, A., Nisha, R., Jagjeeta, K. and Kaushik, C.P. (2005). Impact of long and short term irrigation of a sodic soil with distillery effluent in combination with bioamendments. *Bioresource Technology* **96**: 1860-1866.

Kretschmer, N., Ribbe, L.and Gaese, H. (2000). Wastewater reuse for agriculture. *Technology Resource Management and Development* **2**: 37-64.

Kumar, S. and Gopal, K. (2001). Impact of distillery effluent on physiological consequences in the fresh water teleost, *Channa punctatus*. *Bulletin of Environmental Contamination and Toxicology* **66**: 617-622.

Kumar, S.K., Fating, J.S., Vaidya, A.N., Bal, A.S. (1999). Wastewater management. *Journal of Indian Association for Environmental Management. **27***: 104-116.

Kumar, R. and Singh, R.N. (eds.) (2009). *Municipal Water and Wastewater Treatment*. pp. 56-76. TERI Press, New Delhi.

Lin, C., Arun, A.B., Rekha, P.D. and Young, C. (2008). Application of wastewater from paper and food seasoning industries with green manure to increase soil organic carbon. *Bioresource Technology* **99**: 6190-6197.

Loewus, F.A. (1952). Improvement in anthrone method for determination of carbohydrates. *Analytical Chemistry* **24**: 219-219.

Lopez, A., Pollice, A., Lonigro, A., Masi, S., Palese, A.M., Cirelli, G.L., Toscano, A. and Passino, R. (2006). Agricultural wastewater reuse in southern Italy. *Desalination* **187**: 323-334.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin-Phenol reagents. *The Journal of Biological Chemistry* **193**: 265-275.

Malaviya, P. and Sharma, A. (2011). Impact of distillery effluent on germination behaviour of *Brassica napus* L*. Journal of Environmental Biology* **32**: 91-94.

Marklund, S. and Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry* **47**: 469-474.

Medhi, U.J., Talukdar, A.K. and Deka, S. (2011). Impact of paper mill effluent on growth and development of certain agricultural crops. *Journal of Environmental Biology* **32**: 185-188.

Misra, R.N. and Behera, P.K. (1991). The effect of paper industry effluent on growth, pigments, carbohydrates and proteins of rice seedlings. *Environmental Pollution* **72**: 159-167.

Muentz, K. (1977). Isoenzymes of α-amylase during pod development of field beans. *Phytochemistry* **16**:1491-1494.

Murillo, J.M., Lypez, R., Fernondez, J.E. and Cabrera, F. (2000). Olive tree response to irrigation with wastewater from the table olive industry. *Irrigation Science* **19**: 175-180.

Nagajyothi, P.C., Dinakar, N., Suresh, S., Udaykiran, Y., Suresh, C. and Damodharam, T. (2009). Effect of industrial effluent on the morphological parameters and chlorophyll content of green gram (*Phaseolus aureus Roxb*). *Journal of Environmental Biology* **30**: 385-388.

Nath, K., Singh, D. and Sharma, Y.K. (2007). Combinatorial effects of distillery and sugar factory effluents in crop plants*. Journal of Environmental Biology* **28**: 577-582.

Nath, K., Singh, D., Verma, A. and Sharma, Y.K. (2009). Amelioration of tannery effluent toxicity in radish based on nutrient application. *Research in Environment and Life Sciences* **2**: 41-48.

Nawaz, S., Ali, S.M. and Yasmin, A. (2006). Effect of industrial effluents on seed germination and early growth of *Cicer arientum*. *Journal Biological Sciences* **6**: 49-54.

Ornon, G. and Demalach, Y. (1987). Response of cotton to treated domestic wastewater applied through trickle irrigation. *Irrigation Science* **8**: 291-300**.**

Paliwal, K., Karunaichamy, K.S.T.K. and Ananthavalli, M. (1998). Effect of sewage water irrigation on growth performance, biomass and nutrient accumulation in *Hardwickia binata* under nursery conditions. *Bioresource Technology* **66**: 105-111.

Pandey, S.K., Tyagi, P. and Gupta, A.K. (2007). Physico-Chemical analysis and effect of distillery effluent on seed germination of wheat (*Triticum aestivum*), pea (*Pisum sativum*) and Lady's finger (*Abelmoschus esculentus*). *ARPN Journal of Agricultural and Biological Science* **2**: 35-40.

Pandey S.N., Nautiyal, B.D. and Sharma, C.P. (2008). Pollution level in distillery effluent and its phytotoxic effect on seed germination and early growth of maize and rice. *Journal of Environmental Biology* **29**: 267-270.

Radosevich, S.R., Holt, J.S. and Ghersa, C. (1997). *Weed ecology: Implications for management*. Wiley publishers, New York.

Ramana, S., Biswas, A.K. and Singh, A.B. (2002a). Effect of distillery effluents on some physiological aspects in maize. *Bioresource Technology* **84**: 295-297.

Ramana, S., Biswas, A.K., Singh, A.B. and Yadava, R.B.R. (2002b). Relative efficacy of different distiller effluents on growth, nitrogen fixation and yield of groundnut. *Bioresource Technology* **81**: 117-121.

Ramana, S., Biswas, A.K., Kundu, S., Saha, J.K., Yadava, R.B.R. (2002c). Effect of distillery effluent on seed germination in some vegetable crops. *Bioresource Technology* **82**: 273-275.

Rao, M.V., Paliyath, G. and Ormrod, D.P. (1996). Ultraviolet-B and Ozone induced biochemical changes in antioxidant enzymes of *Arabiodopsis thaliana*. *Plant Physiology* **110**: 125-136.

Rath, P., Pradhan, G. and Misra, M.K. (2011). Effect of Distillery Spent Wash (DSW) and fertilizer on growth and chlorophyll content of Sugarcane (*Saccharum officinarum L.*) plant. *Recent Research in Science and Technology* **3**: 169-176.

Ross, S.M. (1994). Toxic metals: Fate and Distribution in Contaminated Ecosystems. In: M.Ross. (Eds.) Toxic metals in Soil-Plant Systems, pp. 189-235. John Wiley & Sons, New York.

Rousan, L.M. (2007). Farmers' perceptions and adoption of using olive-mill wastewater on barley crop in Jordan. *World Journal of Agricultural Sciences* **3**: 280-284.

Rowe, D. and Abdel- Magid, I. (1995). *Handbook of Wastewater Reclamation and Reuse*. Lewis Publishers, Boca Raton, FL.

Rui, L., Qixing, Z., Lanying, Z. and Hao, G. (2007). Toxic effects of wastewater from various phases of monosodium glutamate production on seed germination and root elongation of crops. *Frontiers of Environmental Science and Engineering in China* **1**: 114-119.

Scandalios, J.G. (1993). Oxygen stress and superoxide dismutases. *Plant Physiology* **101**: 7-12.

Shaaltiel, Y. and Gressel, J. (1986). Multienzyme oxygen radical detoxifying system correlated with paraquat resistance in *Conyza bonariensis*. *Pesticide Biochemistry and Physiology* **26**: 22-28.

Shannon, L.M., Kay, E. and Lew, J.Y. (1966). Peroxidase isozymes from Horseradish roots: Isolation and physical properties. *The Journal of Biological Chemistry* **241**: 2166-2172.

Sharma, D.C. (2005). By order of the court: Environmental cleanup in India. *Environmental Health Perspectives* **113**: A394-A397.

Shatanawi, M. and Fayyad, M. (1996). Effect of Khirbet As-Samra treated effluent on the quality of irrigation water in the Central Jordan Valley. *Water Research* **30**: 2915-2920.

Singh, A.B., Biswas, A.K. and Ramana, S. (2003). Effect of distillery effluents on plant and soil enzymatic activities and groundnut quality. *Journal of Plant Nutrition and Soil Science* **166**: 345-347.

Singh, K.K. and Mishra, L.C. (1987). Effects of fertilizer factory effluent on soil and crop productivity. *Water, Air and Soil pollution* **33**: 309-320.

Singh, P.P., Mall, M. and Singh, J. (2006). Impact of fertilizer factory effluent on seed germination, seedling growth and chlorophyll content of gram *(Cicer aeritenum)*. *Journal of Environmental Biology* **27**: 153-156.

Singh, S., Rekha, P.D., Arun, A.B. and Young, C.C. (2009). Impacts of monosodium glutamate industrial wastewater on plant growth and soil characteristics. *Ecological Engineering* **35**: 1559-1563.

[SOE] INDUSTRIAL POLLUTION - State of the Environment Report. (2003).

<http://parisara.kar.nic.in/PDF/ip.pdf >. Accessed 2012 Mar 5.

Sukanya, T.S. and Meli, S.S. (2004). Effect of Distillery Effluent Irrigation on Growth, Yield and Quality of Maize Grown on Sandy Loam in Northern Transitional Zone of Karnataka. *Karnataka Journal of Agricultural Sciences* **17**: 405-409.

Sundaramoorthy, P., Kunchithapatam, J., Thamizhiniyan, P. and Venkateslu, V. (2001). Effect of fertilizer factory effluent on germination and seedling growth of groundnut varieties. *Journal of Ecobiology* **13**: 03-08.

Tayyar, S. and Yapici, A.N. (2009). Seed germination characteristics of broad bean, lentil and common bean irrigated with different dilutions of pre-tanning effluents. *Interciencia* **34**: 514-517.

Tsadilas, C.D. (1997). Irrigation of corn with municipal wastewater. *Acta Horticulturae* **449**: 699-706.

Umebese, C.E., Ade-Ademilua, O.E. and Olonisakin, B.O. (2009). Impact of Combined industrial effluent on metal accumulation, nitrate reductase activity and yield of two cultivars of *Vigna unguiculata* (L.) walp. *Journal of Environmental Science and Technology* **2**: 146-152.

[USEPA] United States Environmental Protection Agency. (1999).

< http://www.epa.gov/npdes/pubs/ozon.pdf>. Accessed 2012 Feb 1.

[USEPA] United States Environmental Protection Agency. (2000). <http://www.epa.gov/npdes/pubs/carbon_absorption.pdf>. Accessed 2012 Mar 1.

[USGS] United States Geological Survey (2012).

<http://ga.water.usgs.gov/edu/waterdistribution.html>. Accessed 2011 Dec 20.

Wins, J.A. and Murugan, M. (2010). Effect of textile mill effluent on growth and germination of Black gram. *International Journal of Pharma and Biosciences* **1**: 1-7.

Yadav, A., Gopesh, A., Pandey, R.S., Rai, D.K. and Sharma, B. (2007). Fertilizer industry effluent induced biochemical changes in freshwater teleost, Channa striatus (Bloch). *Bulletin of Environmental Contamination and Toxicology* **79**: 588-595.