EFFECT OF INDUSTRIAL EFFLUENT ON THE SEED GERMINATION AND SEEDLING GROWTH OF KIDNEY BEANS

(*Phaseolus vulgaris* L.)

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CERTIFICATION

This is to certify that this final year project was carried out under my supervision, by OMOTUNDE, Mercy Oluwaseyi with the matriculation number BTH/11/0263 in the Department of Plant science and Biotechnology, Federal University Oye Ekiti.

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DEDICATION

I dedicate this report to the Almighty God for making it possible for me to have a wonderful research work.

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With the heart of gratitude, I reference the almighty God my maker for supporting me from the inception of this programme to the end. I appreciate him for his goodness and mercy that endureth forever over my life.

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ABSTRACT

The effect of industrial effluent on the seed germination, seedling growth, stem length, leaf length, biomass, chlorophyll content and proximate analysis of kidney beans was investigated. The experiment was carried out with 0% (control),25%, 50%, 75% and 100% concentrations of each effluent concentration was mixed with 5kg of soil in a plastic pot and each treatment was carried out in three replicates. Germination of seeds begins 5th day after planting (DAP) in control and treated seeds. Germination growth increased in the 25% concentration and thrived better in the 50% than the control whereas there was a decrease in the growth of the plant as the effluents concentration increased to 75% and no germination in100% concentration which was significantly different ($p<0.05$) from that of the control, 25%, 50%, and 75% concentration. This study demonstrated that the lower the concentrations of the industrial effluent caused a positive impact on seed germination, growth and chlorophyll content of *phaseolus vulgaris* L. However at higher concentrations of the effluent, toxic effects were observed. This suggested that the effluent could be used safely for *phaseolous vulgaris* cultivation only after proper treatment.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

Effluents are wastes produced from industries and they vary depending on the human activities that produce them. Production of these wastes is an integral part of industrial activities but unfortunately our inability to anticipate or predict the types and magnitude of undesired consequences of unbridled release of effluents in our environment, coupled with the growth of industrialization have resulted in massive and destructive operations in our ecosystems(Uaboi-Egbenni *el al;*2009). Effluents are discharged into rivers, estuaries, lagoons or sea without any form of treatment. However, despite the treatment being employed by some industries, it is still impossible to remove all undesirable properties from effluents.

Environmental pollution is a major concern and has been accepted as a global problem because of its adverse effects on human health, plants, animals and exposed materials (Irshad *et al.,* 1997). Rapid industrialization, deforestation, oil spillage(Adeyinka and Urum, 2004), exploitation of natural resources, unplanned construction of road and building, drains, services of motor cars, production of metals from ore, sewage, solid wastes, use of chemicals, fertilizers and human population are the major key factors for environmental pollution in this universe(Sharma *et al.,* 2004). The continuous increase in industries has become sources of pollution. These industries include battery manufacturing, iron and steel, plastics, chemicals, fertilizers, textile, food and beverages, breweries, pharmaceuticals, soap, petroleum and petrochemical, automobile, tannery, paper mill and cosmetics, tobacco and paint industries (Brown *et al.,* 1996). Biochemical, biophysical and cellular processes; morphological and genetic adaptations enable living things to survive. Stevens *et al.,* (1998) stated that a useful distinction exists between exists between wastes which pose a potentially high risk to human health and those with much less hazards. Many pollutants such as pesticides, oil, hydrocarbons, heavy metals as well as thermal and radioactive pollutants can get into aquatic environments through direct or indirect release from industries, agriculture and households (Faith *et al.*, 2008).

The dairy industry is one of a major source of waste water (Britz *et al.*, 2006). The milk industry generates between 3.739 and 11.217 million m^3 of waste per year. Waste water is generated in milk processing unit, mostly in pasteurization, homogenization of fluid milk and the production of dairy products such as butter, cheese, milk powder etc. most of the milk processing unit use 'clean in place' (CIP) system which pumps cleaning solutions through all equipment in this order water rinse; caustic solution (sodium hydroxide) wash, water rinse, and sodium hypochlorite disinfectant. These chemicals eventually become a part of waste water (Thompson *and George* 1998). Large amount of water is used to clean dairy plants; hence, the resulting waste water can contain detergent, salts and organic matter depending upon source (Belyea *et al.*, 1990).

1.1 Origin of Kidney Beans

Phaseolus vulgaris, the common bean (Gentry, (1969) (also known as the string bean, field bean, flageolet bean, garden bean, haricot bean, pop bean, or snap bean), is a herbaceous plant grown worldwide for its edible dry seed or unripe fruit that are both occasionally used as 'beans'. Its leaf is also occasionally used as vegetable and the straw as fodder. Its botanical classification, along with other *Phaseolus* species, is as a most of whose members of the legume family Fabaceae, most of whose members acquire the nitrogen they require through an association with rhizobia, a species of nitrogen-fixing bacteria. The common bean is a highly variable species that species have a climbing habit, Phillips, R., Rix, M. (1993). But many cultivars are classified as 'bush beans', depending on their style of growth. These include kidney bean, the navy bean, the pinto bean, and the wax bean. The other major types of commercially grown bean are the runner bean (*Phaseoulus coccineus*) and the broad bean (*Vicia faba*).

Kingdom: Plantae

Order: Fabales

Family: Fabaceae

Subfamily: Faboideae

Tribe: Phaeoleae

Subtribe: Phaseolinae

Genus: Phaseolus

Species: P. vulagris

These large red beans are popular in chili, particularly in the American south. They are in the same family of beans as black beans, pinto beans and navy beans. Like most other dry beans, kidney beans are only eaten cooked. In fact, raw kidney beans (and their sprouts) are actually poisonous. It only a few minute of cooking at high heat to neutralize the toxins, which is much less than any standard cooking time for these beans. Kidney beans are excellent sources of protein, which makes them popular as a meat-substitute for vegetarians. They also have high levels of iron, calcium and magnesium.

1.1.2 Growth and Development

It takes the kidney beans plant ten to fourteen days to germinate in a condition where the light requirement is full, watering is regular and not heavy, the soil condition is loose and well-draining and the container is suitable. It can also be harvested on the 100^{th} to 140^{th} day.

1.1.3 Method of Planting

Unfortunately, kidney beans don't transplant well so it's best to just plant the seeds in your garden when the time is right rather than try to start early with indoor seedlings. Choose a put in the garden that will get full sun, and where the soil is loose enough to allow for good drainage. If you find the water pools up when it rains, it won't be a good spot for your kidney bean plants. Put your seeds out after your last frost date, and cover any sprouted seedlings if you do get an unexpected frost. Space your seeds by about 4 inches if you have a vining variety of bean and a bit farther to 8 inches if you are growing a more compact bush type. Bean seeds should be about an inch to an inch and a half under the soil when you plant.

1.1.4 Soil Requirement for Kidney Bean Production

Kidney bean plants do not have 'wet feet', so they do not need water too often unless the weather has been really dry. Watering of the plant is only essential if the soil has dried out, rather than water to keep it constantly moist. They have the ability to produce their own nitrogen in their roots, they don't really need any extra nutrients of fertilizer. While feeding the plant, it is essential not to use any high-nitrogen mixtures around the beans because it will affect their growth. They may seem to thrive with more leaves, but at harvest time they usually end up with a lot of empty pods. Shallow roots make it difficult to hoe around a bean plant without harming the plant. Weeds should be pulled by hand, or by using a good layer of mulch to keep out the weeds.

1.1.5 Pest and Diseases

Kidney beans have nice large leaves that often fall prey to any number of beetles and grubs in the garden. Various species of bean beetle are the biggest threat, though slugs, cutworms and leafhoppers can all be found in your bean patch. If you check your plant daily and pick up any pests you find, you may not need to spray at all. You will also need to keep off the aphids, which are a bit harder to see than the larger beetles. They usually don't do much harm on their own but they can spread bean mosaic virus, which will kill any plants that are not resistant.

Aside from the bugs, your bean plants can also get rust or mildew on the leaves. Bean rust is a fungus that shows up as rusty reddish-brown patches on the leaves and can be treated with fungicide, provided you start to treat plants before it spreads through the leaves. Powdery mildew is less of a danger, but can kill your plants if you don't keep in check. It just looks like a fine white powder on the leaves that doesn't wipe off. Again, treat with fungicide and keep your plants from being too moist. Humid air helps mildew thrive, so only water your plants at the soil level, not over the leaves.

1.1.6 Harvesting and Storage

1.1.6.1 Maturity Period

The crop requires between 85 and 120 days from planting to maturity depending on variety. The first half of this period is vegetative development and the latter half is reproductive. In vine types there is an overlap of the two periods because continued vegetative growth occurs after flowering begins. Flowering continues for 2 to 3 weeks so there can be new pods, half developed pods and fully developed pods as well as newly opened flowers present on many plants in early August. Pods are initially green changing to light brown or tan as they mature. Each pod can contain 2 to 4 seeds depending upon variety.

1.1.6.2 Mode of Harvesting

They are usually harvested with puller-cutters, followed by drowning and combining. Beans are generally ready for harvest approximately two weeks after bloom. The beans should be harvested just before the seeds are mature and before they form bumps on the pod. The pod should be firm and snap when they are bent. Beans should be picked every 2-3 days to ensure the plants remain productive. They should be pinched rather than pulling to avoid damaging the plant.

1.1.6.3 Storage

Mold development on beans in storage is influenced by the temperature of the beans and by the relative humidity of the air in the spaces. For all grains, 75% relative humidity in theses spaces will provide safe storage, if normal temperatures are maintained.

1.2 Objective of the Research

This research will be carried out to:

i. To determine the effect of industrial effluents of Fanmilk Plc on seed germination, and seedling growth of kidney beans

ii. To determine the protein content of the plant in each treatment

iii. To determine the chlorophyll content of the leaf in each treatment

iv. To determine the fresh and dry weight of the beans plant in each treatment.

1.3 Environment

Industrial wastewater is the effluent discharged by manufacturing units and food processing plants. This is the waste produced by industrial activity which includes and material that is rendered useless during a manufacturing process such as that of factories, mills, and mining operations. It has existed since the start of the industrial revolution (Maczulak, Anne Elizabeth, 2010). Some examples of industrial waste are chemical solvents, paints, sandpaper, paper products, industrial by-product, metals, and radioactive wastes. Toxic waste, chemical waste, industrial solid waste and municipal solid waste are designations of industrial waste. Environmental protection and rational use of natural resources and other industrial raw materials has become an important sphere of mankind's advancement in the 20 the century. Mankind's demand for resources and raw materials has intensified the ecological and economic contradictions in the industries (Sen and Chakraborty, 2009). This wide spread industrialization in urban areas has drastically reduced land area for waste disposal. Disposal of untreated industrial and domestic wastes into the environment affects both soil and ground water quality.

Soil and streams have been used for multifarious purposes including waste disposal. Our careless dumping of wastes has affected these precious resources (Quazilbash *et al.,* 2006). Environment is a system that includes all the living organisms interacting with the physical environment as a whole to maintain a balanced state. Abiotic and biotic factors are the two components, where the abiotic or nonliving portion comprises of the flow of energy, nutrients, water, and gases and the concentrations of organic and inorganic substances. It is characterized by the continuous cycling of matter. The biotic, or living, portion include the three general categories of organisms based on their method of acquiring energy: the primary producers which include the green plants; the consumers, which include all the animals; the decomposers, which include the microorganisms that break down the remains of plants and animals into simpler components for recycling in the biosphere (Mc Naughton and Wolf, 1979).

2.4 Water Pollution

Water pollution has become a serious issue in the present scenario. Various industries are draining their effluents to the rivers even without any treatment. There are also several other pollutants that contaminate the major water sources. Discharge of treated or untreated effluents from various industries is the chief contribution of pollution to the inland surface waters. Seth (1976) explained about the contaminations through industrial effluents, sewages, residues of pesticides, fertilizers and detergents that harmfully affect the environment. The disposal of waste water on land has assumed greater importance in recent days as the volume of waste water production is increasing day by day and the inland water sources are insufficient to accommodate the water expelled out (Bole and Bell, 1978; Sanai and Shayegam, 1979).

The output of industries, agriculture and urban communities generally exceeds the biologic capacities of aquatic systems, causing waters to become choked with an excess of organic substances. When the organic matter exceeds the capacity of those microorganisms in water that break it down and recycle it, the excess of nutrients in such matter encourages the excessive growth of algae leading to the depletion of dissolved oxygen and a series of events takes place and are called as eutrophication (Dhaliwal and Khel, 1995). Round (1979) stated that the eutrophication is mainly due to the increasing level of phosphorus in the aquatic system.

Most of the hazards coming to human and ecosystem are mostly due to ground water pollution. The untreated sewage, industrial effluents and agriculture wastes are often discharged into the water bodies. This contaminated water spread wide range of water borne diseases. The agricultural fields around these water bodies are affected (Chandra and Kulshreshtha, 2004; Tung *et al.*, 2009).

1.5 Utilization of Industrial Effluents

Recycling of industrial effluents is the most effective and economical practice for dealing with the pollution problems. The role of biological organisms in the effective utilization of industrial effluents was identified by several workers (Rani *et al.*, 1990; Marwha *et al.*, 1998; Kumawat *et al.*, 2001; Raina and Aggarwal, 2003; Chandra and Srivastava, 2004). Thabaraj *et al.* (1964) suggested that the utilization of industrial waste for agricultural purpose was a solution to the waste disposal problem. According to Larsen *et al.* (1975) industrial organic wastes if used safely and effectively in proper concentrations can increase the fertility of soil. Though industrial effluents are used for irrigation, these may contain certain toxic substances besides the nutrients that promote the growth of the crop plants (Dolar*et al.,* 1972; Saxena, 2003). Certain industrial effluents such as those of distilleries, fertilizer factories, tanneries etc. have the potential of reutilization and may be harnessed for human welfare. The effluents produced from the distilleries are a rich source of organic matter as well as essential micro and macro nutrients (Samuels, 1980; Kulkarni, 1982; Rani *et al.,* 1990; Om *et al.,*1994; Nemede and Shrivastava, 1996; Chatterjee *and* Chatterjee 2003; Sivaraman and Thamizhiniyan, 2005).

1.6 Effect of Industrial Effluents on Plants

The effluent is an inevitable consequence of industrial process. In arid and semi-arid regions of the country, where shortage of water becomes limiting factor, the effluent is being used for irrigational purposes by the farmers in agriculture and agro-forestry practices. Since the production of wastewater is a continuous process, it can cater for substantial irrigation requirements. This alternative use of wastewater will not only prevent the waste from becoming an environment hazard but also will serve as a potential source of fertilizer if used rationally and at appropriate concentration

Generally speaking, wastewater (treated and untreated) is extensively used in agriculture because it is a rich source of nutrients and provides all the moisture necessary for crop growth. Most crops give higher than potential yields with wastewater irrigation; reduce the need for chemical fertilizers, resulting in net cost savings to farmers. If the total nitrogen delivered to the crop via wastewater irrigation exceeds the recommended nitrogen dose for optimal yields, it may stimulate vegetative growth, but delay ripening and maturity, and in extreme circumstances, cause yield losses. Crop scientists have attempted to quantify the effects of treated and untreated wastewater on a number of quality and yield parameters under various agronomic scenarios. An overview of these studies suggests that treated wastewater can be used for producing better quality crops with higher yields than what would otherwise be possible. The use of untreated municipal wastewater, as is the practice in many countries, pose a whole set of different problems. Nevertheless, the high concentration of plant food nutrients becomes an incentive for the farmers to use untreated wastewater as it reduces fertilizer costs, even when the higher nutrient concentrations may not necessarily improve crop yields. Most crops, including those grown in peri-urban agriculture, need specific amounts of NPK for maximum yield. Once the recommended level of NPK is exceeded, crop growth and yield may negatively be affected. For example, urea plant effluents are a rich source of liquid fertilizer but in concentrated forms they have adverse effects on rice and corn yields (Singh and Mishra, 1987). The composition of municipal wastewater also has to be taken into account. Predominance of industrial waste brings in chemical pollutants, which may be toxic to plants at higher concentrations. Some elements may enter the food chain, but most studies indicate that such pollutants are found in concentrations permitted for human consumption. On the other hand, predominance of domestic wastewater may result in high salinity levels that may affect the yield of salt sensitive crops. This shows that the economic impacts of wastewater on crops may differ widely depending upon the degree of treatment and nature of the crops. From an economic viewpoint, wastewater irrigation of crops under proper agronomic and water management practices may provide the following benefits: (1) higher yields, (2) additional water for irrigation, and (3) value of fertilizer saved. Alternatively, if plant food nutrients delivered through wastewater irrigation result in nutrient over supply, yields may negatively be affected.

Impact from wastewater on agricultural soil, is mainly due to the presence of high nutrient contents (Nitrogen and Phosphorus), high total dissolved solids and other constituents such as heavy metals, which are added to the soil over time. Wastewater can also contain salts that may accumulate in the root zone with possible harmful impacts on soil health and crop yields. The leaching of these salts below the root zone may cause soil and groundwater pollution (Bond, 1999). Prolonged use of saline and sodium rich wastewater is a potential hazard for soil as it may erode the soil structure and effect productivity. This may result in the land use becoming non- sustainable in the long run. The problem of soil salinity and sodality can be resolved by the application of natural or artificial soil amendments. However, soil reclamation measures are costly, adding to economic constraints resulting in losses to crop productivity. Moreover, it may not be possible to restore the soil to the original productivity level, by using these soil amendments. Hence, wastewater irrigation may have long-term economic impacts on the soil, which in turn may affect market prices and land values of saline and waterlogged soils. Wastewater induced salinity may reduce crop productivity due to general growth suppression, at pre-early seedling stage, due to nutritional imbalance, and growth suppression due to toxic ions (Kijne *et al.*, 1998). The net effect on growth may be a reduction in crop yields and potential loss of income to farmers. Wastewater irrigation may lead to transport of heavy metals to soils and may cause crop contamination affecting soil flora and fauna. Some of these heavy metals may bio-accumulate in the soil while others, e.g., Cd and Cu, may be redistributed by soil fauna such as earthworms (Kruse and Barrett 1985). Studies conducted in Mexico (Assadin *et al.,* 1998), where wastewater mixed with river water has been used for crop irrigation for decades, indicate that polluted water irrigation may account for up to 31percent of soil surface metal accumulation and lead to heavy metal uptake by alfalfa. However, heavy metal concentrations in alfalfa pose no risk to animal or human health. The impact of wastewater irrigation on soil may depend on a number of factors such as soil properties, plant characteristics and sources of wastewater. The impact of wastewater from industrial, commercial, domestic, and dairy farm sources are likely to differ widely. The use of dairy factory effluents for 22 years in New Zealand shows that nearly all applied P is stored in the soil while nitrogen storage is minimal, implying nitrogen leaching and consequent nitrate pollution of the groundwater (Degens *et al.*, 2000).

Wastewater is a rich source of plant food nutrients. The impact of wastewater irrigation on yield varies from crop to crop. If the crops are undersupplied with essential plant food nutrients, wastewater irrigation will act as a supplemental source of fertilizer thus increasing crop yields. Alternatively, if plant food nutrients delivered through wastewater irrigation result in over supply of nutrients, yields may negatively be affected. In the absence of any chemical fertilizer application, wastewater nutrients will act as a sole source of fertilizer, delivering savings in fertilizer cost.

2.6.1 Seed Germination

Germination is defined as 'those consecutive events which cause a dry quiescent seed in response to water uptake to show a rise in its general metabolic activities and to initiate the formation of seedlings from the embryo' (Mayer and Poljakoff Mayber, 1989). Bewley (1997) explained germination as 'those events that begin with water uptake by the seed and end with the elongation of the embryonic axis and penetration by the radicle of the structures surrounding the embryo'. Yung (1938) reported that the rice seed germination under favorable conditions starts with the pushing of coleorhiza through the pericarp leaving a cavity in front of the root cap. The primary root elongates and fills the cavity and subsequently the coleorhiza is penetrated. Root extends its growth towards the epicotyl for a short distance before it responds towards gravity. At this time the coleoptile also emerges and grows rapidly. Bewley and Black (1985) emphasized the role of embryonic axis on enzyme mediated reserve food mobilization which controls the germination and subsequent seedling growth.

Garg and Kaushile, (2008) evaluated the suitability of textile mill wastewater (treated and untreated) at different concentrations (0, 6.25, 12.5, 25, 50, 75, and 100%) for irrigation purposes. Effect of textile mill wastewater on germination, delay index, physiological growth parameters and plant pigments of two cultivars of sorghum was studied. The textile effluent did not show any inhibitory effect on seed germination at lower concentration (6.25%). The other reported plant parameters also followed the similar trend. Seeds germinated in 100% effluents did not survive for longer period. It was been concluded that the effect of the textile effluent was cultivar specific and due care should be taken before using the textile mill wastewater for irrigation purposes. Rehman*et al.* (2009) reported that the textile effluent affected seed germination and early growth of some winter vegetable crops. The textile effluent reduced seed germination and early growth of all vegetables. Turnip was observed most susceptible while radish was tolerant to textile effluent treatments. Sasikala and Poongodi,(2013) studied the impact of dye effluent at various concentrations (4%, 8%, 10%, 12% &16%) on seed germination of black gram for a period of fifteen days. She reported gradual decrease in the shoot and root length of the seedlings with the increase in the dye effluent concentrations.

Medhi*et al.* (2008) studied the effect of pulp and paper mill effluents on seed germination and seedling growth of mustard, pea and rice seeds and observed that the lower concentration of effluent with 40%, 50% and 30% were suitable for germination and seedling growth of mustard, pea and rice respectively. The study had also revealed that the germination of seeds and seedling seeds and seedling growth gradually declined with the increasing concentration of the effluent. However, rate of inhibition was reported different for different seeds. The study suggested that the effluent could be used safely for agricultural purpose, if used with proper dilution.

Germination levels have significant influence on vigor, field performance and yield in several crops. The decrease in germination levels lead to a suboptimal population of plants per unit area and reduction in vigor may result in poor performance by the surviving plants. Substantial loss of yield was reported in beans, peas and barley when low rate of germination was recorded in the seeds (Roberts, 1972). In wheat, a reduction in yield was recorded when germination was lowered to about 50% (Agarwal and Dadlani, 1984). Decline in germination levels resulting in reduction in vigour, yield components and yield has been reported in maize (Hussaini *et al.,* 1988), rice (Raghavendr Rao *et al.,* 1990), and sorghum (Rao *et al.,* 1992). Pretorius *et al.,* (1994) studied the germination in *Phaseolus vulgaris* and correlated with mobilization efficiency and respiration of germinated seeds. Tao-Hanzhi *et al.,* (1995) correlated the increased vigour index with the increased water uptake and increased sugar and protein content in the cotyledons whereas Basu *et al.,* (1998) correlated the vigour index with the membrane integrity and the physiological condition of the seed.

The scientific aspect of seed treatment with chemicals were well studied by Kidd and West (1918, 1919) and according to them, the factors that influence the plant during early stages of development may also influence immensely on its subsequent growth. Kirkbly (1968) reported that the reduction in germination percentage may be due to the reduction in the respiratory rate.

The application of sewage and industrial effluents to improve the seed germination and seedling growth were studied by several researchers (Rajaram *et al.,* 1988; Shinde *et al.,* 1988; [illegible] *et al.,* 1999; Arindam 2000; Pandey and Pandey, 2002; Verma *et al.,* 2004).

The effect of distillery and fertilizer factory effluents on seed germination, speed of germination, germination index, seedling growth of *Phaseolus radiatus* and *Oryza sativa* were studied by Sahai *et al.,* (1979, 1983, 1985). They noticed a decrease in the seed germination and speed of germination with increasing concentration and a complete arrest in germination in pure effluent. Treatment with mixed effluent showed a marked variation in germination percentage and seedling growth in jowar, rice and bajra (Somashekar *et al.,* 1984). The diluted effluent showed an increase in germination whereas the effect was reverse in pure effluent. Sisoida and Bedi (1985) studied the impact of chemical industrial effluent on germination and growth of wheat and explained the action of diluted effluent treatment for the promotion in germination.

CHAPTER TWO

2.0 MATERIALS AND METHOD

2.1 Source of Industrial Effluent

The industrial effluents used in this study were obtained from fanmilk Plc. Ibadan, Nigeria where milk base products such as fan vanilla, super yogo and a lot more and the effluent was collected from the main drain which is the main collection point.

2.2 Source of Plant Materials

The kidney beans seeds were obtained from the International Institute for Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria.

2.3 Germination Experiment

5kg of the soil samples were weighed and put inside plastic buckets. The soil in each bucket was treated with 25%, 50%,75% and 100% concentrations of industrial effluent. Another plastic bucket containing soil did not receive any treatment with industrial effluent. This served as control. Each treatment was then replicated three times. 5 seeds of kidney beans were sown into the soil in the plastic buckets. The soil in the plastic buckets were regularly watered to ensure that the soil was moist enough for plant germination.

2.4 Determination of Biomass

Fresh and dry weight of each seedling was determined. This was carried out by measuring the weight of the fresh leaf in each treatment using a weighing balance. The dry weight of the air-dried leaves were also determined. This was done by oven-drying the leaves at 80 ± 5^0C for 48 hours.

2.5 Determination of chlorophyll Content

Newly formed, fully opened leaves from treated soil and control plants were collected for pigment estimation. Chlorophyll a, b and total chlorophyll were determined by extracting one gram of fresh fully expanded leaves in 80% acetone and measuring the colour intensity of the extract at 645 and 663 nm wavelengths using a UV/VIS spectrophotometer. Chlorophyll contents were computed using the formula described by Arnon (1949) as shown below:

Chlorophyll a (mg/mL) = $12.7 A_{(663)} - 2.69 A_{(645)}$

Chlorophyll b (mg/mL) = $22.9A_{(645)} - 4.68 A_{(663)}$

Where;

$A_{(645)}$ = absorbance at a wavelength of 645nm

$A_{(663)}$ = absorbance at a wavelength of 663nm

Total chlorophyll (mg/ml) = chlorophyll a + chlorophyll b.

2.6 Proximate Analysis

2.6.1 Determination of Moisture Content

The sample (10 g) was weighed W_1 into a known weight of an empty petri dish. The weight of the petri dish and the sample was taken and recorded as W_2. The Petri- dish was placed into a preset oven at 105 °C for 3 hours in order to reduce the moisture. After 3 hours, the petri dish was taken out and placed inside a desiccator for 30 minutes to cool. The sample was there weighed W_3 (AOAC, 2005a).

$$Percentage\ Moisture\ Content\ (\%) = \frac{W2-W3}{W1} X \frac{100}{1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (4)$$

2.6.2 Determination of Fat Content

The crude fat content will be determined as reported in AOAC (1990b). Using soxhlet apparatus. A considerable amount of the sample will be put in a pre-weighed thimble, weighed, and dried in an oven. The thimble containing the sample will be place in the receiver of the soxhlet apparatus. Normal hexane BP 60-68°C will be used as solvent for the extraction; a 500 ml round bottom flask will be filled to ¾ with the solvent. The flask will be fitted to the soxhlet apparatus with a reflux condenser and place in an electro mantle heater. The solvent will be heated and refluxed several times and continued for 4hrs until the condenser will detached. The thimble containing the defatted sample was remove and dried to a constant weight in an oven at 100 °C. The difference in weight before and after extraction will record and express as percentage crude fat extracted.

$$Percentage\ Crude\ Fat\ Content\ (\%) = \frac{Weight\ of\ extracted\ fat}{weight\ of\ sample} X \frac{100}{1} \quad \ldots\ldots\ldots\ (5)$$

2.6.3 Determination of Total Ash Content

An amount of 1.0 g of the sample will be weighed in a clean pre-weighed W_1 crucible and the weight will be record as W_2. The crucible with the sample will be place in a muffle furnace and the temperature will be increase to 500 °C for 3hrs in order to allow the sample to burn. The ashing will continue until the sample become grey in appearance. After ashing, the crucible with the ash will cool in desiccator and then weigh as (W_3) as reported in AOAC (1990b).

$$\% \, Total\ Ash\ Content = \frac{(weight\ of\ crucible + ash) - (weight\ of\ crucible)}{Weight\ of\ sample\ before\ ashing} X \frac{100}{1} \quad \text{.... (6)}$$

$$\% \, Total\ Ash\ Content = \frac{W3 - W1}{W2 - W1} \quad \text{... (6.1)}$$

Where:

W_1 is the weight of empty crucible

W_2 is the sample and crucible before ashing

W_3 is the crucible and the ash sample.

2.6.4 Determination of Crude Fibre

Crude fibre is the portion of the plant material which is not ashed or dissolved in boiling solution of 1.25% H_2SO_4 or 1.25% NaOH. The crude fibre will be determined using the standard procedure described by Pearson, *et al.,* (1981). Each defatted sample will be weighed and transferred into a 500 ml conical flask and 200 ml of 1.25% H_2SO_4 will be added and the sample will be boiled for 30mins using cooling fingers to maintain constant temperature. After boiling, the mixture will be pour into filter cloth under gentle suction using a buchner funnel, rinsed well with hot distilled water. The material will be transferred into a conical flask containing 200ml of 1.25% NaOH and boiled for another 30mins while shaking gently to avoid spillage. The sample solution will be filtered, washed with hot distilled water and with 1% HCl respectively. The washing will be repeated twice with ethanol and trice with petroleum ether to remove any remaining fat. The residue will be transferred into a clean, dried crucible, oven dried, cooled in the desiccator and weighed as (W_2). The crucible will be place in the muffle furnace at 450°C for 2 h. cooled in a desiccator and reweighed as (W_3).

$$\% \, Crude \, Fibre = \frac{Weight \, of \, crude \, fibre}{Weight \, of \, sample \, used} \, x \, \frac{100}{1} \quad \text{.. (7)}$$

$$\% \, Crude \, Fiber = \frac{W2 - W3}{W1} \quad \text{.. (7.1)}$$

Where: W_1 is the weight of the sample

W_2 is the weight of sample + crucible after oven drying.

W_3 is the weight of the sample + crucible after ashing.

2.6.5 Determination of Crude Protein

This analysis was carried out in 3 stages;

2.6.5.1 Digestion Stage

This stage involves digestion of the sample. A known quantity (0.5 g) of the sample will be digested with 10 ml H_2SO_4 with 0.5 g selenium as catalyst in a microkjeldahl digestion flask and the mixture will be heated on an electro thermal heater until a clear solution is obtained. The flask will be allowed to cool after which the digest is diluted with distilled water into a 100 ml standard flask. The sample is transferred into the Kjeldahl distillation unit.

2.6.5.2 Distillation Stage

It involves the steam distillation of the digest to which 10ml of 40% NaOH solution is added to release the ammonia. 3 drops of mixed indicator bromo cresol green and methyl red is then added to the receiving flask containing 10 ml of 2% boric acid solution to give a pink colour solution. The sample will be distilled until about 50 ml of the distillate is collected in the receiving flask. A colour change from red wine to green observe will indicating the presence of ammonia.

Equation: Sample + conc. $H_2SO_4 \rightarrow (NH_4)_2SO_4$

$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O$

The collected ammonia forms a complex with the boric acid as

$NH_3 + H_2BO_3 \rightarrow 2NH_4^+ + BO_3^-$

2.6.5.3 Titration Stage

It involves the titration of the resulting solution in the conical flask against 0.1 M HCl solution until a colour change from green to red wine is obtained indicating the end point.

Equation: $NH_4^+ + HCl + H_3O^+ \rightarrow NH_4Cl + 2H_2O$

$$\%\ Nitrogen = \frac{Titre\ value \times Molarity\ of\ acid\ used \times 0.014 \times Dilution\ factor}{Weight\ of\ sample} \times \frac{100}{1} \text{..... (8)}$$

$$\%\ Crude\ Protein = \%\ gN \times 6.25 \text{ .... (8.1)}$$

2.6.6 Carbohydrate Determination

This is the summation of protein, fat, moisture content, ash, crude fibre minus 100.

$$\%\ Carbohydrate = 100 - (Moisture\ content + Fat + Crude\ fibre + Ash + Protein)$$

2.7 Statistical Analysis

In this study, two major variables were analysed using the statistical package for the social sciences (SPSS) version 21:i. the significant difference and ii. Means. The significant difference was calculated at 1% confidence limit using Analysis of Variance (ANOVA), a statistical method for testing the Duncan Multiple Range Technique (DMRT).

CHAPTER THREE

3.1 RESULTS

This chapter presents and discusses the results of the analyses done in this study. In Table 1. Germination of seeds begins 10days after planting in control and treated seeds except in 100% of treated seeds. The percentage of seeds germination varies according to the concentration of the industrial effluent. On the 1st week of planting, germination were recorded in control (0%), 25%, 50%, 75% and 100% concentration respectively. Germination then increased till the 4th week in pot treated with 0%, 25%, 50%, and 75% except in 100% concentration. These values were recorded till the 6th week of planting. From these values, it was observed that the rate of germination was higher even than the control in 50% treated experiment while as the concentration of effluent increased further above this range, the percentage germination reduced and there was no germination at all at 100% treated experiment.

Table 1: Effect of industrial effluent on seed germination of kidney beans seed (cm).

Treatment	Week1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	8.90 ± 0.01^{a}	10.90 ± 0.01^{d}	13.00 ± 0.01^{d}	14.90 ± 0.07^{d}	18.10 ± 0.01^{c}	21.00 ± 0.07^{c}
25%	7.50 ± 0.01^{c}	9.00 ± 0.01^{c}	12.00 ± 0.00^{c}	12.80 ± 0.00^{c}	14.00 ± 0.01^{d}	14.70 ± 0.00^{b}
50%	9.00 ± 0.01^{d}	11.00 ± 0.00^{e}	13.00 ± 0.00^{d}	15.10 ± 0.00^{e}	18.14 ± 0.07^{c}	21.50 ± 0.01^{e}
75%	5.50 ± 0.01^{c}	6.70 ± 0.01^{b}	8.40 ± 0.00^{b}	10.00 ± 0.00^{b}	11.00 ± 0.01^{b}	12.50 ± 0.00^{d}
100%	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.01^{a}	0.00 ± 0.00^{a}

Results are mean of 3 determinations ± SE. Letter a, b, c, d and e within the same Colum signifies that means with different letters differ significantly at $P< 0.05$, while means with the same superscript letter does not differ significantly at $P< 0.05$. A statistical analysis was carried out on the results obtained using statistical package for social science SPSS version 20.0 2010 to determine the mean and Standard error

3.2 Fresh and dry weight

Table 2, shows the effect of the industrial effluent on the fresh weight and dry weight of the seedlings sown in different concentrations of the pollutants and control. The 50% treated experiment has the greater value of fresh and dry weight of 18.50±0.01 and 4.80±0.93 respectively even than the control experiment and the values reduced as the concentration of the effluent increased above 50% concentration.

Table 2: Effect of industrial effluent on fresh and dry weight of kidney beans seed (mg)

Treatment	Fresh weight	Dry weight
Control	13.25 ± 0.01^{c}	3.50 ± 0.39^{c}
25%	16.33 ± 0.88^{d}	3.55 ± 0.40^{c}
50%	18.50 ± 0.01^{e}	4.80 ± 0.93^{e}
75%	9.00 ± 0.58^{a}	3.1 ± 1.33^{b}
100%	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}

Results are mean of 3 determinations ± SE. Letter a, b, c, d and e within the same Colum signifies that means with different letters differ significantly at $P < 0.05$, while means with the same superscript letter does not differ significantly at $P < 0.05$. A statistical analysis was carried out on the results obtained using statistical package for social science SPSS version 20.0 2010 to determine the mean and Standard error

3.3 GROWTH PARAMETER

3.3.1 Leaf length characteristics

The result of the leaf length (cm^2) are shown in table 3. This shows the effect of different concentrations of the effluent on the leaf length of experimental seedlings of kidney beans. Leaf length increased with time (in weeks) in each treatment concentration. The 50% concentration of the treated experiment has the largest leaf length even than the control experiment and the leaf length reduced as the concentration increased beyond this value. The largest leaf length was observed at the sixth week in the 50% treated experiment with a leaf length of $7.60 \pm 0.01 cm^2$ compared with no leaf in the 100% treated experiment which was significantly different ($p<0.05$) from the control and other concentrations.

Table 3: Effect of industrial effluent on leaf length of kidney beans (cm)

ment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
rol	6.10 ± 0.01^{d}	6.20 ± 0.01	6.40 ± 0.01^{d}	6.90 ± 0.00^{c}	7.00 ± 0.01^{c}	7.40 ± 0.00^{c}
[illegible]	4.00 ± 0.01^{c}	4.20 ± 0.01^{c}	5.35 ± 0.01^{b}	5.50 ± 0.01^{b}	5.70 ± 0.01^{b}	5.90 ± 0.01^{b}
[illegible]	0.00 ± 000^{a}	6.40 ± 0.00^{c}	6.90 ± 0.01^{d}	7.00 ± 0.01^{c}	7.40 ± 0.00^{d}	7.60 ± 0.00^{d}
[illegible]	3.10 ± 0.01^{b}	3.50 ± 0.01^{b}	5.00 ± 0.01^{b}	5.40 ± 0.01^{b}	5.00 ± 0.00^{b}	5.70 ± 0.00^{b}
%	0.00 ± 000^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Results are mean of 3 determinations ± SE. Letter a, b, c, d and e within the same Colum signifies that means with different letters differ significantly at $P < 0.05$, while means with the same superscript letter does not differ significantly at $P < 0.05$. A statistical analysis was carried out on the results obtained using statistical package for social science SPSS version 20.0 2010 to determine the mean and Standard error

3.3.2 Number of leaves of kidney bean

Table 4, shows the result of number of leaves of kidney bean in each treatment. The number of leaves in each treatment increased with time (in weeks). The number of leaves increased progressively in 25%n and 50% concentration of the treated experiment even than the control experiment in all weeks, while the number of leaves reduced even to the minimum value in 75% and 100% concentration of the treated experiment. At the sixth week of the experiment, the number of leaves in 25% and 50% concentration of the treated experiment were 30.00±0.01 and 32.00±0.09 respectively, which were greater than the control experiment (25.00±0.01) and the number of leaves of leaves in 100% concentration was zero. In all weeks of the experiment, the maximum number of leaves were observed in 50% concentration of the treated experiment which was significantly different ($p<0.05$) from that of control, 75% and 100% concentration of the treated experiment.

Table 4: Effect of industrial effluent on no of leaves of kidney beans

Treatment	Week1	Week 2	Week 3	Week 4	Week 5	Week 6
Control	2.00 ± 0.01^{b}	6.00 ± 0.01^{e}	16.00 ± 0.01^{d}	20.67 ± 0.34^{d}	22.00 ± 0.01^{c}	25.00 ± 0.01^{c}
25%	2.00 ± 0.01^{b}	4.00 ± 0.01^{c}	20.00 ± 0.01^{c}	25.00 ± 0.01^{e}	28.00 ± 0.01^{e}	30.00 ± 0.01^{d}
50%	3.00 ± 0.01^{c}	5.00 ± 0.01^{d}	13.00 ± 0.01^{c}	18.00 ± 0.01^{c}	23.00 ± 0.01^{d}	32.00 ± 0.09^{e}
75%	2.00 ± 0.01^{b}	3.00 ± 0.01^{b}	10.00 ± 0.01^{b}	12.00 ± 0.01^{b}	13.00 ± 0.01^{b}	15.00 ± 0.01^{b}
100%	0.00 ± 0.01^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Results are mean of 3 determinations ± SE. Letter a, b, c, d and e within the same Colum signifies that means with different letters differ significantly at $P< 0.05$, while means with the same superscript letter does not differ significantly at $P< 0.05$. A statistical analysis was carried out on the results obtained using statistical package for social science SPSS version 20.0 2010 to determine the mean and Standard error

3.3.3 Number of seeds per pod of kidney bean fruit

Table 5, shows the effect of different concentration of the effluents on the number of seeds per pod of the kidney bean fruits. The number of seeds per pods increased progressively with time (in weeks) in each treatment concentration whereas it decreased as the concentration of the pollutant increased except for 50% concentration of the pollutant which has higher number of seeds per pod even than the control. At the sixth week of the experiment, 3.33±0.33 was recorded in 50% concentration of the treated experiment which was significantly different ($p<0.05$) from the control and other concentrations but there was no significant difference between the number of seeds per pod in 25% and 75% concentrations of the treated experiment.

Table 5: Effect of industrial effluent on of seeds per pod of kidney beans seed

Treatment	Week 3	Week 4	Week 5	Week 6
Control	1.67± 0.33^{B}	2.67± 0.67^{BC}	3.67 ± 0.33^{C}	1.67 ± 0.33^{C}
25%	0.00± 0.00^{A}	1.00 ±0.10^{AB}	1.67 ± 0.33^{B}	1.67 ± 0.33^{B}
50%	2.00± 0.00^{B}	3.67± 0.33^{C}	3.31 ± 3.33^{C}	3.33 ± 0.33
75%	0.00± 0.00^{A}	0.00 ±0.00^{A}	1.00 ± 0.37^{AB}	1.33 ± 0.33^{B}
100%	0.00± 0.00^{A}	0.00 ±0.00^{A}	0.00 ±0.00^{A}	0.00 ±0.00^{A}

Results are mean of 3 determinations ± SE. Letter a, b, c, d and e within the same Colum signifies that means with different letters differ significantly at $P< 0.05$, while means with the same superscript letter does not differ significantly at $P< 0.05$. A statistical analysis was carried out on the results obtained using statistical package for social science SPSS version 20.0 2010 to determine the mean and Standard error

3.4 Biochemical Experiment

3.4.1 Chlorophyll content of kidney Beans Leaves

Table 6, shows the effect of the different concentration of the effluent on the chlorophyll content of the freshly harvested leaves. The chlorophyll a, chlorophyll b and total chlorophyll of the leaves in 50% concentration of the treated experiment was found to be significantly different ($p<0.05$) from that of the control and other concentration of the effluent. The leaves with 50% concentration has higher total chlorophyll content even more than the control, while the chlorophyll content reduced as the concentration of the effluent increased beyond 50% concentration.

Table 6: Effect of industrial effluent on Chlorophyll content of kidney beans leaves

Treatment	Chlorophyll A	Chlorophyll B	Total
Control	18.04±0.01^{d}	79.55±0.01^{a}	97.55±0.01^{a}
25%	131.55±0.01^{d}	81.87±0.01^{b}	213.63±0.07^{d}
50%	318.02±0.01^{e}	210.80±0.01^{b}	526.75±0.01^{e}
75%	126.62±0.01^{c}	157.14±0.67^{d}	182.99±0.01^{c}
100%	0..00±0.00^{a}	0.00±0.00^{c}	0.00±0.00^{b}

Results are mean of 3 determinations ± SE. Letter a, b, c, d and e within the same Colum signifies that means with different letters differ significantly at P< 0.05, while means with the same superscript letter does not differ significantly at P< 0.05. A statistical analysis was carried out on the results obtained using statistical package for social science SPSS version 20.0 2010 to determine the mean and Standard error.

3.4.2 Proximate composition of the bean seeds

The effect of different concentrations of the effluent on the proximate composition of the bean leaves is shown in table 7. The 50% concentration of the treated experiment showed higher composition of proximate with 83.82$\pm$0.01 of moisture,11.29$\pm$0.01 of Ash, 19.27$\pm$0.01 of fat, 12.50+0.01 of protein, 25.30$\pm$0.01 of CHO and 7.80$\pm$0.01 of crude fiber which is significantly different from the control and other concentrations.

Table 7: Effect of industrial effluent on proximate composition of the beans seed

Treatment	Moisture	Ash	Fat	Protein	CHO	Crude Fibre
Control	73.68±0.01^{a}	5.13±0.01^{c}	11.10±0.01^{b}	12.43±0.01^{c}	8.60±0.01^{b}	6.25±0.01^{c}
25%	74.40±0.01	4.76±0.01^{a}	14.27±0.74^{b}	4.50±0.01^{a}	17.80±0.01^{c}	4.12±0.01^{e}
50%	83.82±0.01^{d}	11.29±0.01^{d}	19.27±0.74^{d}	12.50±0.01^{c}	25.30±0.01^{e}	7.80±0.01^{d}
75%	82.14±0.01^{d}	5.00±0.01^{c}	9.50±0.01^{a}	8.85±0.02^{b}	22.72±0.01^{d}	3.84±0.01^{c}
100%	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}	00.00±0.00^{a}

Results are mean of 3 determinations ± SE. Letter a, b, c, d and e within the same Colum signifies that means with different letters differ significantly at P< 0.05, while means with the same superscript letter does not differ significantly at P< 0.05. A statistical analysis was carried out on the results obtained using statistical package for social science SPSS version 20.0 2010 to determine the mean and Standard error

CHAPTER FOUR

4.0 DISCUSSION

Effect of industrial effluent was evaluated on germination pattern, leaf length, dry and fresh weight of the kidney beans leaves. The effect on chlorophyll content and the proximate composition of the kidney beans were also investigated. Germination was low in 75% concentration and there was no germination in 100% concentration of the low rate germination was probably due to toxicity resulting from pollutant contamination around the seeds.

The rate of seed germination of kidney beans increases progressively with increasing concentration of 50% and thereafter it decreases as the concentration increases beyond this level (Table 1). The effect of industrial effluent on seed germination of kidney bean seed was at higher concentration did not support the growth of the plant; this is in accordance with the finding of Khan *et al.* (2011) who showed that the germination of seed is affected at higher concentration of textile effluents. Nagda*et al.* (2006) found that, at higher concentration of industrial effluent, the seed germination efficiency decreases. Osmotic pressure of the effluent increases at higher concentrations of total salts making inhibition more difficult and retard germination efficiencies. The ability of seeds to germinate under high osmotic pressure differs with variety as well as species (Unger, 1987). From the experiment, at 50% concentration of the treated experiment, the percentage of seed germination was higher even than the control experiment. This is in accordance with the findings of Agbogidi*et al.*, (2007) who showed that the effect of crude oil pollution on plants is dependent on the level of pollution and that small amount of mineral oils may actually be beneficial to plants. The reduction in seed germination may be due to higher soluble salt in the polluted water. Khan and Sheikh (1976) have reported significant reduction and delay in the germination of *Capsicum annum* seeds with the treatment of sewage. They revealed that, this is due to decrease in water uptake at higher level of salinity in view of toxicity of high osmotic pressure due to high soluble salts.

The result showed that increase in effluent percentage encouraged the decrease in the length of the leaf. We may relate the decrease in leaf length with the elevated amounts of total dissolved solids at higher concentrations. This could also be related to the fact that some of the nutrients present in the effluents are essentials but above a particular concentration, they might become hazardous. Panaskar&Pawar (2011a & b) in his research reported that textile effluents were not inhibitory at low concentrations but with the increase in concentration growth of seedling leaves was affected. Hussain*et al.* (2010) found that tannery effluents caused a reduction in germination, leaves length and growth of sunflower parameters along with other parameters like chlorophyll content, protein and carbohydrate content etc. The results of this study indicated that the effects on leaves length were proportional to the concentrations of the effluents and higher concentration showed stronger effect on the leaves thereby decreasing the leaf length.

Due to toxic effect of the pollutant, there was reduction in fresh and dry weight with increase in concentration of the effluent, except in seedlings treated with 25% and 50% concentration which have increase in dry weight significantly increased with increasing in industrial effluent concentration as compared to that of control plant. The increase in fresh weight varied from 25% waste water to 100 % waste water. Industrial effluent had positive effects on biomass (fresh weight and dry matter). The interactions between kidney beans and industrial effluents treatments on biomass were significant. Dry matter accumulation is an important parameter which tells about the accumulation/deposition of heavy metal and other ions inside the body, which may help in combating the stress.Plant biomass is an indicator of crop productivity in terms of dry matter yield. Increased photosynthetic process is considered a base for the building up of organic matter, which accounts for 80-90% of the total dry mass of plant (Bishnoi*et al.,* 1993a,b).

Number of leaves is an indicator of plant growth and there is a reduction in the number of leaves at higher concentrations of the effluent which significantly reduces the yield of the plant. Similarly, reduction in the number of leaves was recorded by Tripathi*et al.* (1999) in *Albizialebbek* seedlings at higher doses (200 ppm) of Cr (VI). These results are in agreement with the findings of (Shah *et al.*, 2009) where a low dose of Hudiara drain influences the growth while growth reduction was observed at higher doses in *Eucalyptus camaldulensis*.

The results regarding the effect of industrial effluent on the proximate composition of the kidney bean shows that higher concentration of the effluent greatly reduced the proximate composition of the kidney beans. Moderate concentration of the effluent, at 25% and 50% concentration, produced higher percentage of protein, CHO, ash, fiber, fat and moisture. The results regarding protein content revealed red kidney beans to be an excellent protein source containing 25.78±0.77% protein. Present results regarding the protein content corroborated the findings of Salini and Sudesh,(2002) who reported protein content of different bean varieties. The crude fibre content in kidney beans was observed to vary from 3.52 ± 0.01 % in 75 % concentration to 7.80 % in 50 % concentration. These findings are in accordance to Costa *et al.* (2006) who observed crude fibre content of 4.26-8.98% in pea, chickpea and common beans. The ash content of bean seeds varied from 2.44±0.01 % to 11.29±0.01 % which falls within the range of 4-7% as reported for different varieties of beans (Rui*et al.*, 2011).

A level of significant reduction in chlorophyll a, b and total chlorophyll as the concentration of the effluent increased was observed except for 50% concentration which was even higher than the control experiment. Chlorophyll contents serve as a key index of metabolic efficiency of plants for the utilization of absorbed nutrients (Ramana*et al.,* 2002). Results indicated an overall increase in photosynthetic pigment measured in terms of chlorophyll a, chlorophyll b, total chlorophyll compared to control treatment plants. This might have resulted in delay in greening and reduction in number of seedlings developing green pigmentation at high effluent concentrations. Reduction in chlorophyll a and chlorophyll b total chlorophyll was reported earlier in six-day-old mung bean when exposed to tannery effluent containing chromium (Bera*et al.,* 1999). Inactivation of enzymes involved in chlorophyll biosynthetic pathway may contribute to general reduction in chlorophyll content in most of the plants under chromium stress (Shanker, 2005). Decline in the leaf pigment, Chl-b was greater compared to Chl-a as the data revealed an increase in Chl-a/Chl-b ratio in all the treatments over control. Reduction in chlorophyll a, chlorophyll b, total chlorophyll was reported earlier in six-day-old mung bean when exposed to tannery effluent containing chromium (Bera and kanta, 1999). Inactivation of enzymes involved in chlorophyll biosynthetic pathway may contribute to general reduction in chlorophyll content in most of the plants under chromium stress (Shanker*et al.*, 2005).The chlorophyll pigments reduced considerably and the reduction was more prominent due to the nickel toxicity. Behera and Misra (1982) noticed a direct correlation between the effluent concentration and photosynthetic activity. While working with industrial effluent on chlorophyll pigment contents, they observed a reduction in pigment contents in plants treated with high concentrations of effluent, whereas the pigment content increased in the seedlings treated with diluted effluents. Sahai*et al.* (1983, 1985) and Sahai and Srivastava (1987) studied the effect of industrial effluents on chlorophyll content in the seedlings of *Orvza sativa*, *Cajanuscajan*and *Phaseolusradiatus*respectively. They observed an increase in the chlorophyll content in the diluted effluent and in the concentrated effluent the pigment content reduced significantly. Singh *et al.* (2006) observed an increase in pigment content in the lower concentrations of fertilizer factory effluent in gram. However, the results of this study showed that higher concentrations of effluents showed a toxic effect on the chlorophyll of kidney beans.This decline in chlorophyll contents beyond 50 % concentration of treated experiment may be due to the inhibition of enzymes responsible for chlorophyll biosynthesis under the influence of higher concentrations of toxins present in the industrial effluents.

4.1 Conclusion and Recommendation

This study reveals that irrespective of the nature, the industrial effluents could be well utilized for agricultural crops on proper dilution, so as to reduce the lethality of the pollutants. Higher concentration ofindustrial effluents creates serious hazards to plants and eventually to human health. In water scarce countries, reuse of effluents for irrigation of various crops is very effective method to meet the demand of proper water and food supply. It may be further concluded that the higher concentration of released industrial effluent causes many types of inhibitory effects on the germination speed, germination value, plant growth, crop yield accumulation of heavy metals in plants and poor human health. The proper treatment and dilution of the effluent is therefore needed before the disposal and usage of industrial effluent for irrigation purposes.

REFERENCES

Adeyinka, J.S. and Urum, K.C.N (2004). Effect of Hydrogen peroxide on crude oil polluted soil. *Asian Journal of Microbiology, Biotechnology and Environmental Science*. 6(3):125-128.

Agarwal, P.K and Dadlani, M. (1984).Seed quality, storability and yield of wheat as influenced by sprouting and spoilage due to rain.*Seed Res.* 12: 11- 19.

Agbogidi OM, Eruotor PG, Akparabi SO, Effects of time application of crude oil to soil on the growth of maize (*Zeamays* L.) *Research Journal of Environmental Toxicology*. 2007;1 (3): 116-123

Ajmal, M., Khan, A.U., 1983.Effects of sugar factory effluent on soil and crop plants.*Environmental pollution*. 30, 135-141.

Arindam Kumar. 2000. Reclamation of soil polluted by industrial effluents using herbaceous flora. *Adv. Pl. Sci.* 13(2): 427-430

Assadian, N. W., Fenn, L. B., Flores-Ortiz, M. A. and Ali, A. S. (1998). Spatial variability of solutes in a pecan orchard surface-irrigated with untreated effluents in the upper Rio Grande River basin.*Agricultural Water Management*. 42,(2), 143-156.

Barcelo, J, Poschenreider, C. and Gunse, B. (1986). Water relations of chromium VI treated bush bean plants (*Phaseolus vulgaris L cv Contender*) under both normal and water stress conditions. *Journal of Experimental Biology*, 37: 178-187.

Bates, L.S., Waldren, R.P and Teare, I.D. (1973). Rapid determination of free proline for water stress studies. *Pl. and Soil* 39: 205-208.

ehera, B. K. and Misra, B. N. (1982).Analysis of effect of industrial effluent on growth and development of rice seedlings. Environ. Res. 28: 10-20.

Belyea R.L., Williams J.E., Gieseka L., Clevenger T.E and J.R. Brown, (1990).Evaluation of dairy waste water solids as a feed ingredient.*Journal of dairy Science*. 73 (7): 1864-1871.

Bera, A.K. and Kanta.B. (1999).Effect of tannery effluents on seed germination, seedling growth and chlorophyll content in mung bean (*Vignaradiata*).*Journal Environmental Ecology*, 17: 955-961.

Bewley, J. D. and Black, M. (1985). Seeds: *Physiology of Development and Germination.* Plenum Press, New York and London.

Bewley, J.D. (1997). Seed germination and dormancy.*Pl. Cell* 9: 1055-1066.

Bishnoi, N.R., Dua, A., Gupta, V.K. and Sawhney, S.K. (1993b). Effect of chromium on seed germination, seedling growth and yield of peas.*Agric. Ecosyst. Environ.*, 47:47–57.

Bishnoi, N.R., L.K. Chugh and S.K. Sawhney.(1993a). Effect of chromium on photosynthesis, respiration and nitrogen fixation in pea (*Pisumsativum L*) seedlings.*Journal of Plant Physiology*, 142:25–30.

Bole, J.B. and Bell, R.J. (1978).Land application of municipal sewage waste water yield and chemical composition of forage crops.*J.Environ. Qual.* 7: 222-226.

Bond, W. J. (1999). Effluent irrigation- an environmental challenge for soil science.*Australian Journal of Soil Research*.No. 4, pp. 543(13).

Britz, T. J., Van Schalkwyk, C. and Hung, Y. (2006).Treatment of dairy processing wastewaters, waste treatment on the food processing industry.1-28.

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(2):1-12.

Gentry, H. S. (1969).'Origin of the Common Bean, Phaseolus vulgaris'. Economic Botany (New York: New York Botanical Garden Press). 23 (1): 55-69.

Hussain, F., Malik, S.A., Athar, M., N. Bashir, U. Younis, M.U. Hassan and S. Mahmood (2010).Effect of tannery effluents on seed germination and growth of two sunflower cultivars.*Afr. J. Biotech*., 9(32): 5113-5120.

Hussaini, S.H., Ahmed, Z.A. and Dhanraj, A. (1988).The effect of accelerated ageing on germination, vigour and yield of maize.*Seed Res*. 16: 68-74.

Hussaini, S.H., Ahmed, Z.A. and Dhanraj, A. (1988).The effect of accelerated ageing on germination, vigour and yield of maize.*Seed Res*. 16: 68-74.

Irshad, A., Ali, S. and Jan, M.R (1997).Physichochemical studies of industrial pollutants. *Proc. NSMTCC97 on Environ.Poll.Islamabad, Pakistan.*

Irshad, A., Ali, S. and Jan, M.R. (1997). Physicochemical studies of industrial pollutants. Proceedings of the NSMTCC on environmental pollution, Islamabad, Pakistan. Pp. 1-96.

Joshi, U. N., Rathore, S. S. and Arora S. K. (1999).Effect of chromium on growth and development of cowpea (*Vignaunguiculata L*).*Indian Journal of Environmental Protection*. 19: 745-749.

Khan, M.G., Danlel, G., Konjit, M., Thomas, A., Eyasu, S.S., Awoke, G. (2011). Impact of textile waste water on seed germination and some physiological parameters in pea

(*Pisumsativum L.*), Lentil (*Lens esculentum L.*) and gram (*Cicerarietinum L.*).*Asian Journal of Plant Science*, 10, 269-273.

Khan, S.S. and Sheikh, K.H. (1976). Effects of different level of salinity on seed germination and growth of *Capsicumannam*. *Biologia.* 22, 15-25.

Kidd, F. and West, C. (1918).The Physiological predetermination. The influence of physiological condition of the seed upon the course of subsequent growth upon the yield. *Ann. Appl. Biol.* 5: 1-10.

Kidd, F. and West, C. (1919).The influence of temperature on the soaking of the seeds.*New Phytol.*18: 35-39.

Kijne, J, W.; S. A. Parthaper; M. C. S. Sahrawat. (1998). How to manage salinity in irrigated lands: A selective review with particular reference to irrigation in developing countries. SWIM Paper 2, International Water Management Institute, Colombo: Sri Lanka.

Kirkbly, E.A. (1968). Influence of ammonium and nitrate nutrition on the anionic balance and nitrogen and carbohydrate metabolism of white mustard plants grown in dilute nutrient solutions. *Soil Sci.* 81: 105-141.

Kruse, E. A. and Barrett, G. W. (1985).Effects of municipal sludge and fertilizer on heavy metal accumulation in earthworms.*Environmental Pollution* (Series A). . 38, 235-244.

Kulkarni, D.P, (1982). Generation of energy and fertilizer from distillery spent wash. *Maharastra Sugar* 7(4):69-72.

Kumawat, D.M. Tuli, K, Singh, P and Gupta, V. (2001). Effect of dye industry effluent on germination and growth performance of two rabi crops. *J. Ecobio*. 13(2): 89-95.

Larsen, W.L., J. Gillery and D.R. Linden.(1975). Consequences of waste disposal on land.*J. Soil-Water Conserv*. 30: 68-71.

Maczulak, A.E. (2010). Pollution: Treating Environmental Toxins. New York: infobase Publishing. P.120.

Marwha, S.S., P.S. Panesar and B/ [ingh.(1998). Studies on the isolation of efficient yeast strain for the treatment of dairy waste water.*Poll. Res.* 17(1): 51-56.

Marwha, S.S., P.S. Panesar and B/ [ingh.(1998). Studies on the isolation of efficient yeast strain for the treatment of dairy waste water.*Poll. Res.* 17(1): 51-56.

Mayer, A.M and Poljakoff- Mayber, A. (1989).*In The germination of seeds.* Pergamon press, Oxford.

McNaughton, S.J and Wolf, L.K. (1979).*General Ecology*. Holt, Rinehart and Winston. New York.

Medhi, U.J., Talukdar, A.K. and Deka, S., (2008). Effect of pulp and paper mill effluents on seed germination and seedling growth of mustard (Brassica campestris), pea (Pisumsativum) and rice (Oryza Sativa) seeds.*Poll Res,* 27(3), 423-442.

Nagda, G.K., Diwan, A.M. and Ghole, V.S., (2006). Seed germination bioassays to assess toxicity of molasses fermentation based bulk drug industry effluent. *Electronic Journal of Environmental, Agricultural and Food Chemistry,* 5(6), 1598-1603.

Nemede N. Pravin and V.S. Shrivastava, (1996).Distillery waste characteristics in satpura region, India.*Poll. Res.* 15(3): 245-249.*Res.* 22(4): 601-604.

ıofe, O., Aremu, M. O. and Akintayo, T. E. (2006).A comparative study on the chemical and amino acid composition of some Nigerian under-utilized legume flours.*Pakistan Journal of Nutrition,* 5(1): 34-38.

ı Hari, Nepal Singh and Manyank S. Arya.(1994). Combined effect of wastes of distillery and sugar mill on seed germination, seedling growth and biomass of okra (*Abelmoschusesculentus* (L.) Monench).*J. Environ. Biol.* 15 (3): 171-175.

ıasker, D.B. and Pawar, R.S. (2011a). Effect of textile mill effluent on growth of *Vignaunguiculata* and *Pisumsativum* seedlings.*Indian Journal of ScienceTechnology,* 4(3): 266-272.

ıasker, D.B. and Pawar, R.S. (2011b). Effect of textile mill effluent on growth of *Sorghamvulgare* and *Vignaaconitifolia* seedlings.*Indian Journal of ScienceTechnology,* 4(3): 273- 278.

ıdey, A.K and Pandey, G.C. (2002).Impact of coal washery effluent on seed germination, seedling growth and chlorophyll content of *Oryza sativa. J. Int. Poll. Cont.* 18(2): 175-181.

illips,R.; Rix, M. (1993).Vegetables. New York: Random House.

ıshanthi, V., K. JeevanRao, A. SreeenivasaRaju and M.V. Shantharam.(1999). Effect of polluted well water and soils on germination and dry matter yield of crops.*Poll. Res.* 18 (1): 67-70.

retorius, J. C., Small, J. G. C and Fagerstedt, K. V. (1998).The effect of soaking injury in seeds of *Phaseolus vulgaris*.L. on germination respiration and adenylate energy charge. Seed Sci. Res. 8: 17-28.

uazilbash AA, Farayal R, Naqui KB, (2006), Efficacy of indigenous Bacillus species in the removal of chromium from industrial effluent. Biotechnology, vol.5 (1), 12-20.

aghavendrRao, D.V.S., Hussaini, S.H., Reddy, B.M. and Ankaiah, R. (1990).Effect of accelerated ageing on germination and yield in rice (*Oryza sativa*. L.). *Proc. lot. Symp. Rice research: New Frontiers. Directorate of Rice Research, Hyderabad.*411-412.

ahman, Abida.,Bhatti, Nawaz. Haq.andAthar, Habib-ur-Rahman., (2009). Textile effluents affected seed germination and early growth of some winter vegetable crops: A case study. *Water Air Soil Pollution*. 198: 155-163.

aina, A.K and Aggarwal, B. (2003).Effect of paper mill effluent on the germination, growth and yield *of Lens esculenta*Moench. *J. Int. Poll. Cont.* 19 (2): 301-306.

ajaram, N., Manoharan, M. and Janardhanan, K. (1988).Effects of Alcohol and Chemical effluents on seed germination and seedling growth of black gram.*Curr. Sci.* 10: 559-560.

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

Rani Renu, M.M. Srivastava and I.P. Saxena.(1990). Effect of distillery waste on seed germination of *Pisumsativum* Lin. *Ind. J. Environ.Hlth.* 32(4): 420- 422.

Rani, R and Srivastava, M.M. (1990).Ecophysiological response of *Pisumsativum* and *Citrus maxima* to distillery effluents. Int. *J. Ecol. Environ. Sci.* 16 (2-3): 125-132.

Rao, R.Y.V., Reddy, B.M., Ankaiah, R and Babuk, G.R.S.S. (1992).Relationship between initial germination and field performance in sorghum seed.*Tech. News* 22(1): 67.

Roberts, E.H. (1972). Loss of viability and crop yields. *In: Viability of seeds. E.H.* Roberts (ed.). Chapman and Hall, London. .307-320

Round, F.F. (1979).*The Ecology of Algae*.Cambridge Publications.

Sahai, R. and Srivastava, N. (1987).Effect of distillery wastewater on the performance of *Cicerarietinum* (L).*Environmental Pollution,* 43: 91–102.

Sahai, R., Shukla, N., Jabeen, S. and Saxeena, P.K. (1985). Pollution effect of Distillery waste on the growth behavior of *Phaseolusradiatus* L. *Environ. Poll.* 37: 245-253.

Sahai, R., Shukla, N., Jabeen, S. and Saxena, P. K. (1983).Pollution effect of distillery waste on the growth behaviour of *Phaseolusradiatus L. Environment Pollution*, 37: 245–253.

Samuels, G. (1980). Rum distillery wastes: potential agricultural and industrial uses in Puerto Rico. *Sugar J.* 43(4): 9-12.

Sanai, M. and Shayegam, J. (1979): Water pollution Abatement through reuse of municipal waste Biol. 27(1): 153-156.

Sasikala, T and Poongodi. N. (2013)., Impact of Dye Effluent on Seed Germination of Black gram (VignaMungo.L.hepper). *Indian Journal of Applied Research*.3(8).

athyanarayana, S., Jarpalapura, N., Thangaraj, J., Madineni, M., &Pullabhatla S. (2011).Characterisation of germinated fenugreek (Trigonellafoneum-graecum L.) seed fractions.*Int J Food Sci Tech*, 46, 2337-23.

axena, A. (2003). Irrigational impact of match factory effluent on *Vignamungo* L. var. Tq. *Poll.*

eth, G.K. (1976). Know your environment. *Sci. Reporter* 13: 7-11.

hah, F.R., Ahmad, N., Masood, K.R., Peralta-Videa, J.R., Zahid, D.M. and Zubair, M. (2009).Response of *Eucalyptus camaldulensis* to irrigation with the Hudiara drain wastewater. *International. Journal of Phytoremediation*, In Press.

halini, H. and Sudesh, J. (2002).Effect of soaking and germination on nutrient and anti nutrient contents of fenugreek (Trigonellafoenumgraecum L.). J *Food Biochem*, 27, 165-176.

hanker, A.K. Carlos, C., Herminia, L. and Avudainayagam, S. (2005). Chromium toxicity in plants.*Environ. Int.*, 31:739–753.

harma, D.C., Sharma, C.P. and Tripathi, R.D. (2003).Phytotoxic lesions of chromium in maize.*Chemosphere*, 51: 63–68.

harma,R., Chandreshwar, L., Kulshreshtha, D., Prasad, F.M., Susan, P. and Kumar, A. (2004). Human exposure to trace elements.*Indian Journal of Environmental Protection.* 24(2):256-260.

Shinde, D.S, R.K. Triwedy and S.D. Khatavkar.(1988). Effect of pulp and paper mill and sugar factory waste on germination of gram (*Ciceraerientinum*).*Poll. Res.* 7(3-4): 117-122.

Singh, K. K.; and L. C. Mishra.(1987). Effect of fertilizer factory effluent on soil and crop productivity.Water, Air and Soil pollution. Vol. 33, pp. 309-320.

ngh, P.P, Manisha, M and J. Singh.(2006). Impact of fertilizer factory effluent on seed germination, seedling growth and chlorophyll content of gram (*Ciceraeritenum*). J. Environ. Biol. 27(1): 153-156.

soda, G.S and S.J. Bedi, (1985).Impact of chemical industry effluent on seed germination and early seedling growth performance of wheat.*Ind. J. Ecol*. 2: 189-192.

varaman, M and P. Thamizhiniyan.(2005). Effect of sago factory effluent on biochemical and mineral contents of black gram (*Vignamungo*).*J. Ecotoxicol. Environ. Monit*. 15(2): 117-122.

mashekar, R.K., Gowda, M.T.G., Shettigar, S.L.N. and Srinath, K.P. (1984).Effects of industrial effluents on crop plants.*Ind. J. Environ. Hlth.* 26: 136- 146.

eevens, J.A., S.S. Vansal, K.W .Khallies, S.S. Knight, C.M. Cooper and W.H. Benson, (1998).Toxicological evaluation of constructed wetland habitat sediments utilizing *Hyalellaazetca* 10-day sediment toxicity test and bacterial bioluminescence. Chemosphere, 36: 3167-3180.

ao-Hanzhi, Cheng-Zhuyu, Tao-Qian, Tao, H.Z., Cheng, Z.Y. and Tao, Q.A., (1995).Effects of some exogenous hormones and microelements on germination and physiological and biochemical changes of tea seeds.*ActaAgronomica-Sinica*. 21(4): 442-450.

habaraj, G.J., S.M. Bose and Y. Nayudamma, (1964).Utilization of tannery effluents for agricultural purposes.*Environ. Hlth.* 6: 18-36.

hompson T. G and George E., (1998) Waste management issues for dairy process state of Wisconsin/department of natural resources. 1-10.

Tokalioglu, S. and Kartal, S. (2006). Statistical Evaluation of the Bioavailability of Heavy Metals from Contaminated Soil to Vegetables.*Bull. Environ. Contam.Toxicol.*, 76:311–319.

Tripathi, A.K., Sadhna, T. and Tripathi, S. (1999).Changes in some physiological and biochemical characters in Albizialebbek as bio-indicators of heavy metal toxicity.*Journal of Environmental Biology*, 20:93–98.

Uaboi-Egbenni, P. O. , Okolie, P. N., Adejuyitan, O. E., Sobande A. O. and O. Akinyemi, O. (2009). Effect of industrial effluents on the growth and anatomical structures of *Abelmoschusesculentus* (okra).*African Journal of Biotechnology*. 8 (14), 3251-3260.

Ungar, L.A., (1987). Halophyte seed germination.*Botanical Review,* 44, 233-264.

Vajpayee, P., Rai, U.N., Ali, M.B. Tripathi, R.D., Yadav, V. and Sinha, S. (2001). Chromium induced physiological changes in *Vallisneriaspiralis L* and its role in phytoremediation of tannery wastewater. *Bull. Environ. Contam.Toxicol*., 67(2):246–256.

Verma, H.K., Tenguria, R.K and Saluja, D.S. (2004).Effect of oil mill effluent on seed germination, early seedling growth and chlorophyll content of mustard seedlings.*Nat. Environ. Poll. Technol.* 3(1): 113-115.

Zurayk, R., Sukkariyah, B. and Baalbaki, R. (2001).Common hydrophytes as bioindicators of nickel, chromium and cadmium pollution.*Water Air Soil Pollution*, 127:373–388.