



## Morphological and Biochemical Analysis of *Cicer arietinum* L. under Paper Industrial Effluent Stress conditions

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**Abstract:** To study the effect of paper industrial effluent on chickpea (*Cicer arietinum* L.) along with different concentration (10%, 20% 40%, 60% 80% and 100%) and pure tape water as a control to compare the effect of paper industrial effluent for 7 days. Physico-chemical characteristics of paper effluent were analyzed in terms of pH, colour, order temperature, DO, BOD, COD, Total hardness, carbonated hardness. All the parameters were found to be higher than the WHO prescribed discharge limits for effluent. The amount of carbohydrate, protein and reducing sugar were comparable with control, their amount were increased at 40% in effluent treated seeds. The chlorophyll content was increased simultaneously with effluent concentration. From this study it is clear that the industrial effluent rich in organic matter and plant nutrients are finding their use in agriculture as cheaper way of disposal.

**Keywords:** Paper industrial effluent, Physico-chemical characteristics, *C. arietinum*

### INTRODUCTION

Industrialization is an important tool for the development of any nation. Today it has become a matter of major concern in the deterioration of the environment; with the rapid growth of industries the industrial waste water has increased tremendously (Amathussalam *et al.*, 2002). Agriculture is the backbone of any economy has also been adversely affected by upsurge in the indiscriminate dumping and disposal of the wastes into land and watercourses. If excessive amount of these nutrition are absorbed by plants, the yield may get reduced and plant may die due to phytotoxicity (Singh and Sakal, 2001). Water resources are affected by industrial pollution extremely. Pollution caused by industrial effluents is a serious problem in throughout the world. The paper industrial effluent is having a higher amount of organic and inorganic elements. They contain higher contents of total hardness, total dissolved solids, biological oxygen demand, chemical oxygen demand, calcium, magnesium, sodium, iron and sulphate (Malik *et al.*, 2014). Paper industrial effluent that has not been treated properly has an unpleasant odor when discharged into the environment. The effluents not only affect the plant growth but also degrade the soil properties when used for irrigation (Patel *et al.*, 2004). So it is necessary to conduct experiments to find out the impact of these effluents on agricultural crops before they are recommended for irrigation. A laboratory experiment was designed to know the effect of different concentration (0-

100%) of paper industrial effluent on seed germination, growth, and biochemical content and yield of chickpea.

### MATERIALS AND METHODS

The effluent was analyzed for its various physico-chemical parameters as per the method of American Public Health Association (APHA, 1992). The industrial effluent was collected and then stored in dark room for future use. The paper industrial effluent was analyzed on *C. arietinum* with different concentration (10%, 20%, 40%, 60%, 80% and 100%) at the germination and seedling growth after 7 days. The petriplates were irrigated with equal volumes of various concentration paper industrial effluent. Control was irrigated with equal volume of distilled water.

#### Physico-chemical analysis

**Estimation of dissolved oxygen (DO):** the paper effluent water sample were collected without bubbling in a 125 ml BOD bottles, add 1 ml of 36.4% MnSO<sub>4</sub> and 1ml iodine azide solution then the reagent is discharge below the surface. 1ml concentrated H<sub>2</sub>SO<sub>4</sub> was added and shaken for completely dissolved, the brown precipitate was formed. Pipet the 50ml sample into a 500ml conical flask and titrate with 0.05N Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> until the iodine color become faint (Carpenter, 1965).

**Estimation of biological oxygen demand (BOD):** Take 125 ml effluent sample in BOD bottles without bubbling. DO was estimated in one bottle by titration method and another bottle were added 1ml of 2% CaCl<sub>2</sub>,

2% $MgSO_4$  and 2% $FeCl_3$ . Bottles were kept for incubation at 27°C for 3 days and then DO was estimated in remaining bottles. BOD was estimated in mg/ml.

**Estimation of chemical oxygen demand (COD):** take 50ml of effluent sample and 5ml of 0.1N  $K_2Cr_2O_7$  was added in each flask yellow orange color appeared and then kept in water bath at 90-100°C temperature for 15-20 min. 5ml of KI solution and 10ml of conc.  $H_2SO_4$  in flask was added, blackish red color appeared. Then titration is done with 0.1N  $Na_2S_4O_6$  until the pale yellow color appeared. 1ml of starch solution were added in flask solution becomes blue. Then again titrated with 0.1N  $Na_2S_4O_6$  until blue color disappear.

**Estimation of total hardness:** 100ml of effluent sample taken in a conical flask. Add 3ml ammonia buffer solution and 0.2 to 0.4 gm of solid Eriochrome black T indicator. Titrated with standard EDTA red to blue and the amount of EDTA solution added is noted in ml. Hardness was expressed in mg/ml.

#### Morphological analysis

**Effect of industrial effluent on seed germination:** For the analysis of seed germination and seedling growth, seed of uniform size, shape, color and weight were selected. Now seed were sterilized with  $HgCl_2$  solution, washed thoroughly with distilled water and kept in different concentration of industrial effluent separately. Seed simultaneously kept in distilled water, which constituted as control. After requisite imbibitions of 42 hours, germination was assessed by radical emergence (2-3mm).

**Effect on 7 days seedling growth:** Seeds were imbibed in different concentration of industrial effluent for up to 24 hours and were grown in separate petri plate for up to 7 days for germination. After 7 days, root shoot separated out from each other by cutting for the analysis. Five plants were collected from each treatment and were analyzed to determine the morphological parameters such as shoot length, root length, fresh weight and dry weight of the crop plant. The number of seeds germinated in each concentration was calculated.

#### Biochemical analysis

**Estimation of total carbohydrate:** Take 10mg sample and 5ml of 2.5N  $HCl$  is added. Boiled in water bath up to 2-3 hours, cool at room temperature. Add 5ml of 2.5N  $HCl$  neutralized with sodium carbonate until the effervescence ceases. The volume is makeup up to 10ml by distilled water and centrifuged at 5000rpm for 5 minutes. Now, 0.2ml of supernatant was taken and add 0.8ml of distilled water, 4ml of Anthrone reagent was added and the solution is heated for 8 minutes in boiling water bath. Cooled rapidly in a freeze and then take O.D. at  $A_{630nm}$  (Hedge *et al.*, 1962).

**Estimation of reducing sugar:** Take 50mg of dry sample and crushed in phosphate buffer. Centrifuge at 5000rpm for 5min, collect the supernatant and divided in to 3 replicates, makeup the volume 3ml by adding phosphate buffer. Add dinitrosalicylic reagent (DNS)

in each replicate and placed in water bath at 100°C for 5-10minutes up to development of orange color. Cooled at room temperature and take O.D at 540nm (Bernfeld, 1955).

**Estimation of total protein:** Take 50 mg fresh plant sample was digested with 8ml phosphate buffer and centrifuged at 5000 rpm for 5min. the 50 $\mu$ l of supernatant was taken from 3 replicates and 2.45ml of 0.1N  $NaOH$  was added then 2ml of reagent A was also added and incubated at 37°C for 30 minutes. After incubation 5ml of reagent B is added and again incubated at 50°C for 5minutes and then O.D. at 750nm is noted. Calibration curve of BSA was useful for the determination of amount of protein (Lowry *et al.*, 1951).

**Estimation of chlorophyll content:** Take 50mg of leaf were crushed in 5ml of chilled acetone (80%) and centrifuged for 5 minutes. After centrifugation supernatant was collected and makeup final volume 5ml by acetone, then O.D was taken at two different wavelength at 645 nm and 663 nm (Arnon *et al.*, 1949).

## RESULTS AND DISCUSSION

Analysis of different parameter of paper industrial effluent as mentioned in table 1. The paper industrial effluent was brownish in color with slightly acidic in nature (pH 6.5) and odor of the effluent sample is very pungent. DO of effluent was 218.4 mg/L. BOD and COD of effluent were 110.4 mg/L and 411.8 mg/L respectively. The total hardness and carbonated hardness of effluent were 456.471 mg/L and 232.94 mg/L respectively.

The germination percentage of 24 hours treated effluent grown seeds are compared with the control plants. The germination percentage is decreased with increasing effluent concentration. No germination is found at 100% concentration of industrial effluent. The lowest percentage of germination is found in the 80% concentration of effluent treated seeds. The higher concentration of effluent is disrupted germination and seedling growth (Nagajyoti *et al.*, 2008). The increase in germination percentage due to the reduction in level of toxic metabolites by dilution and better utilization of nutrients present in the effluent (Kannan, 2001). The reduction in germination percentage at higher concentrations may be due to the excess amount of minerals and nutrients present in the effluent (Kumar, 1999; Singh *et al.*, 2006; Baskaran *et al.*, 2009 and Sasikali and Poongodi,

**Table 1.** Physico-chemical properties of paper industrial effluent.

S.N.	Parameters	Reffluent
1.	Color	Brownish
2.	Odour	Pungent smell
3.	pH	6.5
4.	Dissolved	218.4 mg/L
5.	Biological Oxygen Demand	110.4 mg/L
6.	Chemical Oxygen Demand	411.8 mg/L
7.	Total hardness	456.47 mg/L
8.	Carbonated Hardness	232.94 mg/L

**Table 2.** Biochemical parameters used on root, shoot, residual cotyledon (RC) and imbibed seed (IS) at different concentration of paper industrial effluent.

Parameters	Planlets	Control	10%	20%	40%	60%	80%
Dry Weight (gm)	Shoot	0.055	0.082	0.115	0.030	0.025	0.010
	Root	0.144	0.135	0.099	0.060	0.035	0.021
	R.C.	0.723	0.792	0.597	0.574	0.354	0.340
Fresh Weight (gm)	Shoot	1.335	1.448	0.776	0.765	0.685	0.432
	Root	0.483	0.663	0.160	0.285	0.148	0.840
	R.C.	2.292	1.032	1.582	1.651	1.492	0.854
Carbohydrate (mg/gm)	Shoot	0.56	0.26	0.41	0.69	0.35	0.18
	Root	0.26	0.18	0.27	0.24	0.47	0.75
	R.C.	2.66	1.63	5.00	3.12	3.39	0.75
	Imbibe Seed	2.07	2.44	2.25	1.54	1.09	0.75
Reducing Sugar (mg/gm)	Shoot	0.60	0.50	2.20	2.20	2.60	3.32
	Root	3.80	3.45	3.94	4.32	4.39	4.54
	R.C.	1.50	1.40	1.60	2.30	2.32	2.40
	Imbibe Seed	0.83	0.80	0.81	0.98	0.81	0.99
Total protein (mg/gm)	Shoot	1.16	3.60	3.56	5.52	3.08	2.89
	Root	4.08	0.48	0.42	0.40	0.76	0.76
	R.C.	1.84	0.46	0.62	0.40	0.60	0.46
	Imbibe Seed	1.32	1.42	1.52	1.62	1.48	1.32
Chlorophyll (mg/gm)	Total Chl	0.00220	0.00230	0.00402	0.00431	0.00469	0.00260
	Chl a	0.00096	0.00096	0.00229	0.00234	0.00276	0.00153
	Chl b	0.01263	0.01268	0.02602	0.02778	0.02971	0.01651

2013). Reduction in seed germination percentage at higher concentration of effluent may be due to the higher amount of solids present in the effluent, which causes changes in the osmotic relationship of the seed and water the reduction in the amount of water absorption take place with results in to reduction of seed germination due to enhanced effluent salinity. (Chandrasekar *et al.*, 1998; Priya Kaushik *et al.*, 2005; and Sing *et al.*, 2005).

The root length of *C. arietinum* seedlings were reduced in lower concentration of industrial effluent as compared to control. The maximum root length is observed 7.4 cm in control and the minimum root length 4.73 cm observed at 80% concentration of industrial effluent. In case of shoot, the maximum shoot length is observed 6.11 cm at 60 % concentration and minimum shoot length 5.10 cm at 80% concentration. The reduction in shoot and root growth at higher concentration of effluent due to less amount of oxygen which might have restricted the energy supply and retarded the growth and development (Kumar, 2000 and Baskaran *et al.*, 2009).

The maximum fresh weight (FW) of shoot and root of *C. arietinum* seedlings is measured 1.44 gm and 0.66 gm respectively at 10% concentration and the minimum FW of shoot and root 0.43 gm and 0.84 gm respectively at 80% concentration of industrial effluent, While in case of residual cotyledons (RC) maximum FW is measured 2.29gm in control and the minimum FW of RC 0.85 gm at 80% concentration of industrial effluent. The maximum dry weight (DW) of shoot *C. arietinum* seedlings is measured 0.11 gm at 20% concentration and the minimum DW of shoot 0.01 gm at 80% concentration of industrial effluent. But in case of root maximum DW is measured 0.14 gm in control and

the minimum DW of root 0.02 gm at 80% concentration of industrial effluent. In RC maximum DW is measured 0.79 gm at 20% concentration and the minimum DW of RC 0.34 gm at 80% concentration of industrial effluent. The reduction in DW of plant materials may be due to the poor growth under effluent irrigation (Baskaran *et al.*, 2009 and Malik *et al.*, 2014). The seedling fresh weight and dry weight increased in the lower concentration and decreased in higher concentration of paper industrial effluent (Indra and Ravi mycin, 2009; Sasikali and Poongodi, 2013).

The maximum carbohydrate content in shoot of *C. arietinum* is observed 0.69 mg/gm at 60% concentration and minimum carbohydrate content in shoot 0.18 mg/gm at 80% concentration of industrial effluent. But in root maximum carbohydrate content is observed 0.75 mg/gm at 80% concentration and minimum in root 0.18 mg/gm observed at 10% concentration of industrial effluent. In RC and Imbibe seeds (IS) minimum carbohydrate content are observed 0.75 mg/gm at 80% concentration and maximum carbohydrate content in RC and IS is 5.00mg/gm and 2.44 mg/gm observed at 20% and 10% concentration of industrial effluent respectively. Total carbohydrate content is higher in waste water irrigated crops than control (Bamniya *et al.*, 2010).

The maximum reducing sugar content in shoot, root, RC and IS of *C. arietinum* are observed 3.32 mg/gm, 4.54 mg/gm, 2.4 mg/gm, and 0.99 mg/gm at 80% concentration and minimum reducing sugar content in shoot, root, RC and IS are observed 0.6 mg/gm, 3.45 mg/gm, 1.4 mg/gm and 0.8 mg/gm at 10% concentration of industrial effluent.

The maximum protein content of *C. arietinum* in shoot

and IS are observed 5.52 mg/gm and 1.62 mg/gm at 40% concentration and minimum protein content of shoot and IS 1.16 mg/gm and 1.42 mg/gm observed in control. While the maximum protein content in root and RC are observed 4.08 mg/gm and 1.84 mg/gm in control and minimum protein content in root and RC 0.4 mg/gm and 0.4 mg/gm observed at 40% concentration of industrial effluent. The increases in protein content of chickpea at lower concentration of industrial effluent were observed (Baskaran *et al.*, 2009 and Jolly *et al.*, 2012).

Chlorophyll estimation is one of the important biochemical parameter which is used as the index of production capacity. According to our results as mentioned, paper industrial effluent also affect the biosynthesis of chlorophyll a and b content, maximum reduction in 80% concentration which retard the growth of plant as well as leaf development. But in lower doses the chlorophyll content show increased amount and approximately double increment was observed in 60% concentration of effluent. Reduction in chlorophyll content induced by higher concentrations of effluent may be associated with mineral ions (Dutta and Boissay, 1997; Pragasam and Kannabiran, 2001; Singh *et al.*, 2006; Baskaran *et al.*, 2009 and Jolly *et al.*, 2012). It may also due to the formation of enzymes chlorophyllase which is responsible for chlorophyll degradation (Neelam and Sahai, 1988).

## Conclusion

The findings of the present study revealed that the paper industrial effluent at lower concentrations (10-40%) promotes the growth and yield of *C. arietinum* but the higher concentrations reduced the same though phytotoxic nature of effluent. It can be concluded that the untreated paper industrial effluent reduced the crop growth. However, the stimulation of growth and yield were observed at lower concentration (10-40%) of effluent. It is suggested that paper industrial effluent have to be diluted up to 40% level before it is used for irrigation. After dilution, the effluent characteristics will become within the prescribed limits and pollution load of the effluent decreased. The effluent at lower (40%) concentration can serve as a liquid fertilizer for the cultivation of agricultural crops.

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