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Impact of paper mill effluent on *Solanum tuberosum* growth and analysis of organic components

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Abstract
In the present study an attempt has been made to access the impact of Amalai Paper Mill effluent on *Solanum tuberosum* growth and analysis of organic components. The presence of many ions in the effluent, retards the water absorption and other metabolic activities this factor plant water stress occurres whenever the loss of water in transpiration exceeds the rate of absorption. The plant water stress reduces photosynthesis along with the reduction of the leaf area. A significant decrease in total chlorophyll as effluent concentration higher than 20%. Reduction in chlorophyll content may also be due to the osmotic effect of high effluent concentration which reduce the uptake of mg, K and other mineral ions, thereby inhibition the synthesis of pigment and effective photosynthetic activities.

Keywords: Amalai paper mill effluent, *solanum tuberosum*, growth, organic components

1. Introduction

Pulp and paper produced corresponds to only 40-45% of original weight of wood, the effluent are heavily loaded with organic matter the utility potential of paper mill effluent for irrigation of crop field has been a controversial proposition due to contradictory reports obtained on the effects of various effluents on crop plant response, (Narwal *et al.*, 2005) [1]. Effluents released from pulp and paper mill contains large amount of heavy metals which get accumulated in plant and soil. They cause huge damage to plants and biological systems (Chandra *et al.*, 2010, and Mehta and Bhardwaj, 2012) [2-3] and even to ground water quality and soil (Balakrishnan *et al.*, 2005, Tripathi *et al.*, 2014) [4-5]. In some studies characteristics of effluents of industries and agronomic properties of various crops have been determined (El-Sawaf, 2005 and Iqbal *et al.*, 2013) [6-7]. The black liquor from pulp and paper mill is a complex colloidal solution of various organic and inorganic polymeric substances like lignin, carbohydrate and their derivatives.

The volume and chemical nature of paper mill effluent depends on the type of manufacturing process adopted. Per kilogram of paper production discharges about 270-450 liters of effluent, along with approximate 50 g. of lignin. Contrary to this the small paper mills without soda recovery discharge 300-400 liters of black liquor effluent containing 200-250 gm. lignin per kg. (Garg and Kaushik, 2008) [8]. It is cultivated in tropical, semitropical temperate climate. It attains height between 25-50 cm and is highly rich in protein content. It is erected; bushy plant grows in warmer regions of India. During present study the impact of paper mill effluent on soil characteristics, morphological response, protein and carbohydrate content on certain crop plants growth has been studied.

2. Material and Methods

1. Sugar: 100 mg of seed powder of 500 mg dry plant material macerated in 80% alcohol. The extract was then centrifuged and supernatant was taken for sugar. The supernatant was then made up to 10 ml. 100 mg of glucose was dissolved in 100 ml distilled water. From this 10 ml solution was again diluted upto 100 ml. So 1 ml of this solution contains 100 μg of glucose (1 mg = 1000μg). From this 0.1, 0.2 to 1 ml were taken inj sepatete test tubes. In each case volume was made upto 1 ml by adding distilled water. In another test tube 0.1 ml of seed or plant material extract was taken and volume was made upto 1.0 ml by adding distilled water. To all these solutions 5 ml of anthrone reagent was added. The solution ware then heated for 7 to 8 minutes in a water bath.

Anthron reagent: 100 mg of anthron was dissolve in 75 ml of AR conc. H_2SO_4

2. **Non-Sugar:** Non-sugar first of all made free of sugars by treatment with 80% ethanol.

It is then extracted with 52% perchloric acid by constant stirring. To the extract enthrone reagent is added and the color develop is measured at 625 nm using reagent blank as standard.

Procedure

0.1 gm seed powder or 500 mg plants material of the overn dried finely ground material into a centrifugal test tube. 2 drops of 80% alcohol added for mixing and the 5 ml of hot 80% C_2H_5OH. Stir thoroughly and let it settle and centrifuge for 5 minutes. Decant the alcoholic solution and repeat the alcoholic extraction procedure by adding 30 ml of hot 80% C_2H_5OH to the residue. The residue left in centrifuge tube is then considered free of sugars. From the extraction of starch and 5 ml of water to the above said test material and the 6.5 ml of 52% $HClO_5$ by contant stiriing. Then added 20 ml of distilled water and centrifuge. Pour the liquid into a 100 ml volumetric flask and added 5 ml of water to the residue.

The extraction was repeated with $HClO_5$ for the next 30 minutes. Wash the contents of the tube with water and diluted to 100 ml and filter. Dilute the alquot portion to give a final concentration equivalent to about 100 μg glucose per ml. To analyse the extract a standard aquesh solution containing 100 μg glucose per 1 ml prepared as sugar estimation.

4. Protein

Macerated 0.1 gm seed powder or 0.5 gm plat material in 20% trichloro acetic acid and centrifuge. Dissolved the precipitate in 0.1 N NaOH and centrifuge. Supernatant was make up to 50 m, 0.1 ml protein solution was added to a 10 ml test tube. Added 5 ml of reagent (3), mix well and allow to stand 10 minute after this 0.5 ml of reagent (4) was added with instantaneous and vigorous mixing after 30 minutes at room temperature. The sample was read at 660 nm. Similarly treated blank was prepared for the zero setting (OD value of 0.50 gives a protein value of 200)

Reagests

- 2% Na_2CO_3 in 0.1 N NaOH
- Fehling solution A and B
- 50 ml of solution (1) and 1 ml of solution (2) are mixed immediately before use.
- 1.0 ml of folin ciocalteu reagent is added to 1.36 ml of water to give a solution of 1 N in acid.

Chlorophyll

1.0 gm leaf is macerated in 15 ml of 80% acetone and one pinch of $CaCO_3$ and acid washed sand is added to facilitate grinding. Mixture is then centrige at 1000 ppm. Supernatant made up to 25 ml with 80% acetone. Read the intensity of color at 649 nm and 665 nm against an acetone blank on spectrophotometer.

Amount of chlorophyll was calculated as given formulae:

- Total chlophyll = 6.45 O.D. at 665 nm + 17.72 O.D. at 649 nm
- 2- Chlorophyll a = (11.63 O.D. at 665 nm - 2.39) O.D. at 649 nm.
- 2- Chlorophyll B= (20.11 O.D. at 649 nm. - 5.18) O.D. at 665 nm.
- Equation I, II and III are used to calculate chlorophyll concentration directly assuming no conversion to pheophytins.

Ascorbic acid

1.0 gm plant material is extracted with water. From this solution the impurities are precipitated and removed by adding neutral lead acetate. The ascorbic acid is precipitated as lead salt by bringing the filtrate to a pH 7.6 with ammonia. The lead salt of the precipitate is heated with H_2SO_4, lead is removed as lead sulphate. Now solution is concentrated under vaccum and further purified to remove other foreing matter, by fractional precipitation with organic solvents *viz*. acetone, alcohol, supernatant was make upto 10 ml 4 ml of above solution was taken to a 10.0 ml test tube and added 0.5 ml of Folin cio calteu reagent with vigorous mixing. After 20 minutes at room temperature, the sample was read at 640 nm. Similarly blank was prepared for the zero setting (0.05 O.D. at 640 nm is equivalent to 50 of ascorbic acid.)

4. Results and Discussion

In case of 10 days old *Solanum tuberosum* plants which are irrigated by 10% and 20% effluents, the percentage concentration of sugar, non-sugar, protein, ascorbic acid chlorophyll a and b have the same result as control 20.8 gm, 22.3 gm, 1.8 gm., 17.2 mg, 555.50 mg and 183.00 mg/100 gm respectively. The percentage concentration of sugar yield at 30%, 50%, 80% and 100% of the effluent among the irrigated plants comes to 20.3 gm, 19.4 gm, 18.7 gm and 18.6 gm/100 gm. respectively. In case of non-sugar, percentage concentration showed decreasing order as the percentage concentration of effluent increased with respect of control as mentioned above. The percentage concentration of protein at 30%, 50%, 80% and 100% yielded 1.6 gm, and 1.5 gm, when compared with control. 1.8 gm/100 gm. by increasing the concentration of the effluent, it was found that the percentage concentration of ascorbic acid further goes up at 17.1 mg, 17.3 mg and 17.3 mg/100 when compared with control 17.2 mg/100 mg. In case of chlorophyll a and b, from 30% to 100%, concentration percentage concentration of chlorophyll a and b have further gone down from 550.13 mg and 178.52 mg to 454.15 mg and 154.34 mg/100 mg respectively when compared with the control levels as mentioned above.

In case of 25 days old *Solanum tuberosum* plants which are irrigated by 10% and 20% effluent, the percentage concentration, measures of sugar, non-sugar, protein, ascorbic acid, chlorophyll a and b are the same with respect of control ones at 21.30 gm, 33.50 gm, 1.30 gm, 34.00 mg, 525.00 mg and 175.65 mg/100 respectively. The percentage concentration of above substances except ascorbic acid have further gone down with the increasing concentration of effluent from 30% to 100% which result in measures 21.15 mg 33.25 gm, 1.30 gm, 510.15 mg and 168.58 mg/100 gm to 20.90 gm, 34.50 gm, 1.95 gm, 425.02 mg and 140.84 mg/100 gm, respectively when compared with control ones as mentioned above. The percentage concentration of ascorbic acid has further gone up with increasing percentage concentration of effluent from 30% to 100% which yield 33.95 mg to 34.95 mg/100 gm in respect of control 34.00 mg/100 mg.

In case of 40 days old *Solanum tuberosum*, plants which are irrigated with 10% and 20% of the effluent, the percentage concentration of sugar, non-sugar, protein, ascorbic acid, chlorophyll a and b are the same in respect of control ones at 2.06 gm, 2.15 gm, 3.55 gm, 30.15 mg, 500.04 mg and 166.89 mg/100 gm respectively. But from 30% to 100% effluent the percentage concentration of above said substances except ascorbic acid have further gone down to 2.0 gm, 1.70 gm, 3.40 gm, 482.12 mg and 160.92 mg to

1.74 gm, 0.95 gm, 2.12 gm, 402.93 mg and 134.53 mg/100 gm respectively. The percentage concentration of ascorbic acid has ruther gone up from 30.00 mg to 32.75 mg/100 with the increasing the percentage concentration of the effluent in respect of control ones at 30.15 mg/100 gm.

The presence of many ions in the effluent, retards the water absorption and other metabolic activities Rajannan and Oblisami (1979) [9], Sahai and Shrivastava (1986) [10], Shrivastava and Sahai (1987) [11], Malla and Mohanty (2005) [12], Chandra *et al.* (2009) [13], Mehta and Bhardwaj (2012) [14] and Tripathi *et al.* (2014) [15].

Table 1: Analysis of 10 days old *Solanum tuberosum* plants irrigated by different percentage of effluents

% of effluent	Sugar (g)	Non-sugar (g)	Protein (g)	Ascorbic Acid (mg)	Chl-a(mg)	Chl-b (mg)
10	20.6	22.8	1.8	16.8	555.50	183.00
20	20.4	22.6	1.8	16.9	555.50	183.00
30	20.3	21.5	1.6	17.1	550.13	178.52
50	19.4	21.4	1.6	17.1	550.09	165.95
80	18.7	20.6	1.5	17.3	484.34	155.92
100	18.6	20.5	1.5	17.3	454.15	154.34
Control	20.8	22.3	1.8	17.2	555.50	183.00
r	-0.98	-0.94	-0.89	0.94	-0.94	-0.98
SD	±0.89	±0.97	±0.14	±0.20	±44.26	±13.19

Table 2: Analysis of 25 days old *Solanum tuberosum* plants irrigated by different percentage of effluents (per 100 g)

% of effluent	Sugar (g)	Non-sugar (g)	Protein (g)	Ascorbic Acid (mg)	Chl-a (mg)	Chl-b (mg)
10	21.30	33.50	1.30	34.00	525.00	175.65
20	21.30	33.50	1.30	34.00	525.00	175.65
30	21.15	33.55	1.30	33.95	510.15	168.58
50	21.15	33.80	1.45	34.25	490.00	158.85
80	20.98	33.80	1.55	34.25	60.75	152.25
100	20.90	34.00	1.95	34.95	425.02	140.84
Control	21.30	33.50	1.30	34.00	525.00	175.65
r	-0.98	-0.96	-0.94	0.88	-0.61	-0.99
SD	±0.16	±0.46	±0.16	±0.38	±181.17	±13.92

Table 3: Analysis of 40 days old *Solanum tuberosum* plants irrigated by different percentage of effluents (per 100 g)

% of effluent	Sugar (g)	Non-sugar (g)	Protein (g)	Ascorbic Acid (mg)	Chl-a (mg)	Chl-b (mg)
10	22.60	32.15	1.75	10.15	500.04	166.89
20	22.60	32.15	1.75	10.15	500.04	166.89
30	23.00	34.70	1.60	10.35	482.12	160.92
50	24.00	34.75	1.52	11.00	460.92	153.84
80	24.50	35.00	1.45	12.25	432.42	144.41
100	24.74	35.50	1.30	12.75	402.93	134.53
Control	22.60	32.15	1.75	10.15	500.04	166.89
r	-0.87	-0.93	-0.92	0.93	-0.99	-0.99
SD	±0.45	±0.51	±0.69	±1.27	±39.13	±13.03

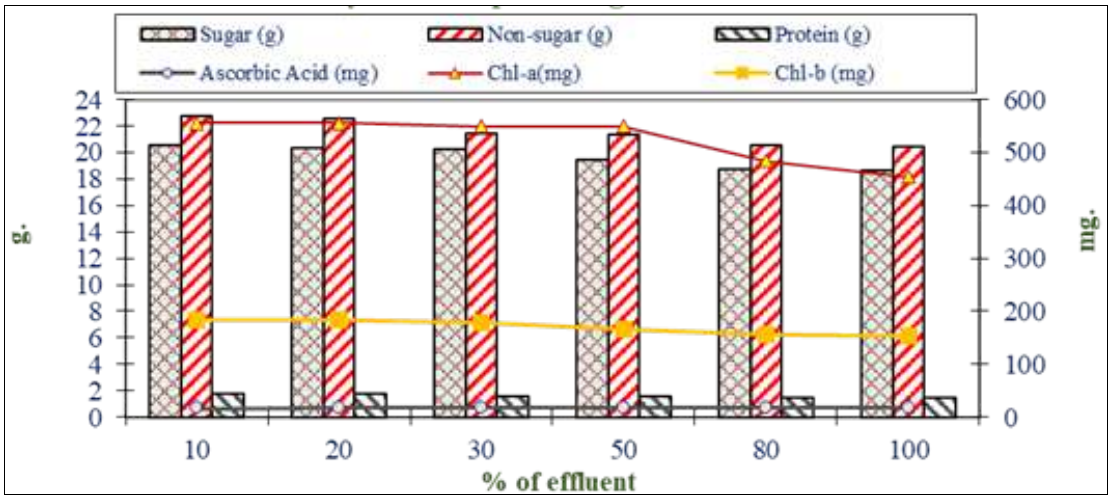

Fig 1: Analysis of 10 days old Solanum tuberosum plants irrigated by different percentage of effluents

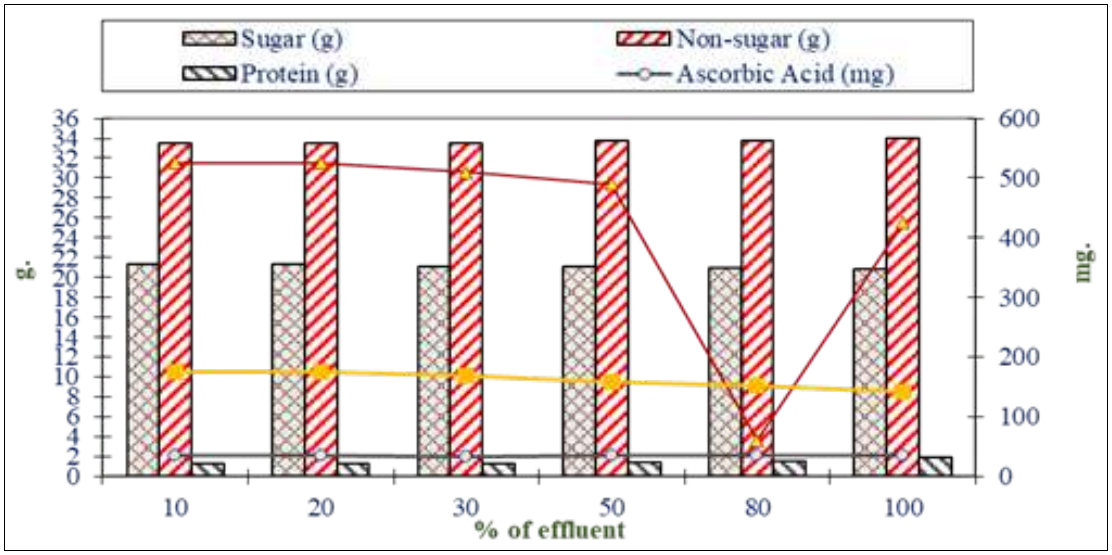

Fig 2: Analysis of 25 days old Solanum tuberosum plants irrigated by different percentage of effluents (Per 100g)

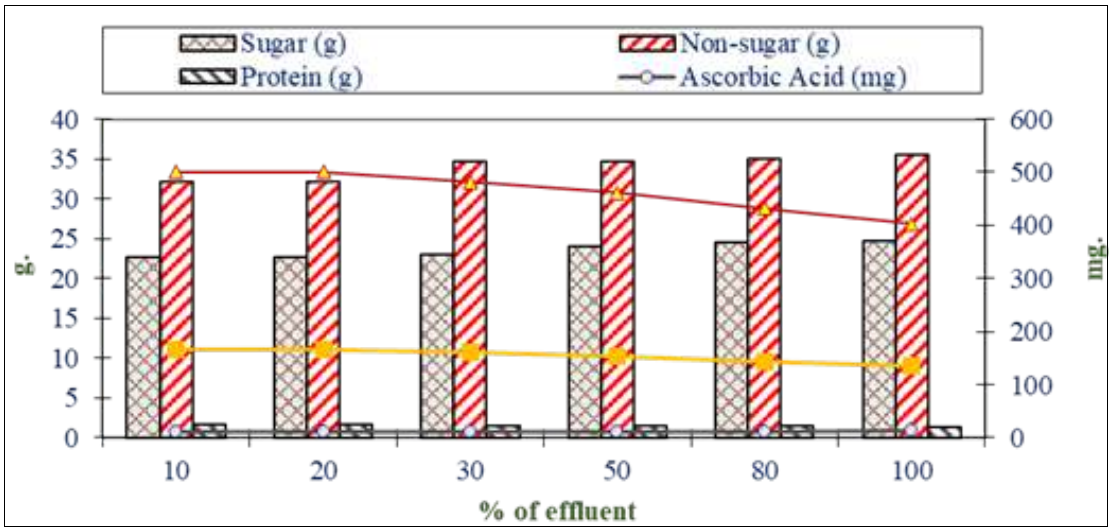

Fig 3: Analysis of 40 days old Solanum tuberosum plants irrigated by different percentage of effluents (Per 100g)

5. Conclusion

Due to this factor plant water stress occurres whenever the loss of water in transpiration exceeds the rate of absorption. The plant water stress reduces photosynthesis along with the reduction of the leaf area. Paper industry effluents are highly polluted industries in India. Small and large scale pulp and paper mills which have different production capacity as well as different raw materials, adopt different processes that lead to radical differences in the physico-chemical properties of effluents. Such polluted effluents must be treated properly before being discharged into the drainage channel, to minimize the effect of various pollutants on the environment.

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