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Impact of paper mill effluent on growth characteristics of *Vigna Mungo* T-9

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Abstract
The present paper deals to Impact of paper mill effluent (PME) was analyzed on black gram (*Vigna mungo* T-9.). Selected parameters selected were chlorophyll, protein content, root length, shoot length, leaf area and total biomass of *V. mungo* T-9. Effects of paper mill effluent were analyzed at different dilutions viz. 5%, 10%, 20%, 40%,30%, 50%, & control. At more than 10% of effluent concentration decline was reported in biomass, protein and chlorophyll (4.9 mg/g highest). However, protein content was reported highest (23.5 mg/g) at 20% of concentration of effluent and showed decline further at higher concentration. Simultaneously soil samples were analyzed which showed, gradual increase in N, PO_4, Na, K, Mg, and Ca at increasing concentration of paper mill effluent. Biochemical parameters like chlorophyll declined (2.5 mg/g) and protein declined (9.1 mg/g) clearly above twenty percent of effluent concentration which resulted in decrease in root length, shoot length, leaf area and biomass of *Vigna mungo* T-9.

Keywords: Effluent, chlorophyll, protein, biomass, leaf area, soil and black gram

1. Introduction

Paper industry is mainly categorized into three types according to their production capacity and raw materials usage i.e. small scale, medium scale and large scale. Paper industry is the sixth largest polluter after oil, cement, leather, environment (Ali *et al.* 2001) [2]. 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 and Madan and Pertibha, 2013) [23, 17]. 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, Kathirvel, 2012 and Mehta & Bhardwaj, 2012) [5, 12, 18] and even to ground water quality and soil (Senthilkumar *et al.*, 2011, Chopra & Srivastava, 2011, Tripathi *et al.*, 2014) [29, 6, 34] and even cancer risk to communities Soskolne *et al.*, 2010) [33]. In agriculture, irrigation water can affect soil characteristics and agricultural crop growth (El-Sawaf, 2005) [8]. In some studies characteristics of effluents of industries and agronomic properties of various crops have been determined (Sawaf, 2005, Mendoza *et al.*, 2006, Bhargava *et al.*, 2008, Iqbal *et al.*, 2013) [28, 19, 3, 11]. 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 50g. 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 Modi, 1999) [10]. Biomethanated and partially treated effluent promotes growth in certain wetland plants (Singh *et al.*, 2012) [31]. *Vigna mungo* T-9 commonly known as black gram is an important crop in Indian subcontinent. 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. The objective of the study is to analyze the effect of paper mill effluent on soil characteristics, morphological response, protein and carbohydrate content of *V. mungo var* T-9 at different effluent concentrations.

2. Material and Methods

The paper mill effluent was collected near Amalai District Anuppur (M.P.). Immediately after collection, the effluent was brought to the laboratory with proper storage for analysis of physico-chemical parameters.

The physico-chemical parameters of paper mill effluent were analyzed by standard methods described by APHA (2005) [1]. Chemical properties of soil (taken in different pots) were analyzed by standard methods described by Trivedy and Goel (1986) [35]. Whereas heavy metals were analyzed by Inductively Coupled Plasma- Optical Emission Spectrophotometer after digestion process.

The pH of the sample was measured by calibrating digital pH meter (Systronics MK6) with buffer solution of pH 4 and 9**.** Turbidity was measured by turbidity meter using the technique of nephelometry, which measures the amount of light scattered at right angles to an incident light beam by particles present in a fluid sample (Sadar 2003) [26].

Total dissolved solids (TDS) of the sample were determined by Hanna Pocket TDS meter. (HI -98130). Conductivity of the effluent sample was measured with a probe and conductivity meter. A voltage is applied between the two electrodes in the probe immersed in the sample water. The drop in voltage caused by the resistance of the water is used to calculate the conductivity per centimeter, as per the manual, by using potassium chloride and distilled water. Dissolved Oxygen is the oxygen concentration in the sample which is defined as the number of milliliters of di-oxygen gas (O_2) per liter of water. DO in effluent sample was measured by Winklers titration. Biological Oxygen Demand (BOD) was measured after five days BOD at 20 ºC was determined by standard dilution technique according to standard methods. Chemical Oxygen Demand (COD) was measured by Potassium dichromate reflux method.

Nitrate Determination is done by spectrophotometer at a wavelength of 480 nm Cataldo *et al.*, (1975) [4]. Inorganic Phosphate estimation in effluent and soil was done by Vanado Molybdophosphoric Acid Method. Ammonia of the effluent was determined as Total Kjeldal Nitrogen (TKN) by Kjeldal method (1883) [13]. Chlorides of the samples were estimated by silver nitrate solution, Sodium, Potassium, Calcium and Magnesium in effluent and soil were measured by Flame Photometer. Alkalinity of the sample was measured by EDTA titration method. Salinity of the effluent was measured by salinity meter Wattson (WSSAs 287).Total hardness was estimated by EDTA titration. For heavy metal i.e. Cu, Cd, Cr, Pb and Zn in effluent and soil samples were analysed by ICP-OES spectrophotometer.

In order to study the effect of paper mill effluent on *V. mungo* growth 21 pots of 20×16 cm height were filled with approximate 2 kg of garden loam soil. Seeds were sterilized by 0.1% mercuric chloride and soaked and germinated. Ten samplings were sown in each pot. The pots were irrigated with different concentration (0.00%, 10%, 20%, 30%, 40%, and 50%) of effluent. These pots were irrigated by 250 ml of effluent of each concentration with 2 day intervals throughout the study period. Seeds sown in pots filled by garden soil and irrigated with sterilized tap water served as control. Regarding germination of seeds emergence of radical as noted as criterion of germination of seed. Number of seeds germinated during stipulated time frame was expressed as percent of germination. After 30 days 3 plants were taken from each set and washed twice by sterilized water. Shoot and root length were measured by meter scale. Biomass of plant was determined by dry weight method as described by APHAs (2005) [1], where plant after harvesting, plant roots, shoots and leaves were separated and dried at 65 C^0 in hot air oven for 48 hours. The difference of fresh weight and dry weight was recorded as biomass of plants.

2.1 Analysis of morphological and biochemical parameters

For pigment determination, 500 mg of dry leaf were homogenized in 20 ml of 80% acetone using mortar and paste and centrifuged at 6000×g for 15 minutes finally the supernatant was made up to 20 ml and Optical Densities (O.D.) were measured at 480 and 510 nm wavelength for carotenoids and 645 nm and 663 nm for chlorophyll on a UV-VIS spectrophotometer (Systronics Model 119, India). The amount of chlorophyll a and b and carotenoid were calculated by using the formulae give by Elcey and Tiwari (1991) [7] respectively.

$$Chlorophyll\ a\ \left(\frac{mg}{g}dry\ leaf\right) = \frac{[12.3 \times D_{663} - 0.86 \times D_{645}] \times V}{d \times 1000 \times w}$$

$$Chlorophyll\ a\ \left(\frac{mg}{g}dry\ leaf\right) = \frac{[19.3 \times D_{645} - 3.6 \times D_{663}] \times V}{d \times 1000 \times w}$$

$$Total\ Chlorophyll\ \left(\frac{mg}{g}dry\ leaf\right) = Chlorophyll\ a + Chlorophyll\ b$$

Where; V = Volume of extract (ml), d= length of light path (cm), w = dry weight of leaf.

Protein content was estimated by Lowry *et al.* (1951) [16]. Testing was done as per the manual and absorbance was measured at 660 nm against blank. Then standard curve was made by taking concentration of protein along X-axis and absorbance at 660 nm along Y-axis.

While Leaf area of black gram seedlings was measured by leaf area meter (Systronics 128).

3. Results and Discussion

It is evident from Table 3, that paper mill effluent was alkaline in nature having pH 7.4. The values of TDS, BOD, COD exceeded the permissible limits of ISI 1982 (IS: 2296), Observed BOD were very high, 294.9 mg/l (Table 1). As compared to the normal values this indicates the high concentration of organic material in effluent. This work is also supported by the work of (Medhi *et al.,* 2011 and Madan & Pertibha, 2013) [20, 17]. COD is more realistic parameter which indicates the pollution status of water body. COD values were quite high 701.2 mg/l (Table 1) As per Indian standards maximum values of COD for pulp and paper mill effluent is 300mg/l. Phosphate, Nitrate and Total Kjeldhal Nitrogen were also found high (710, 357, 487 mg/l respectively).

Heavy metals in the effluent were found to be exceeding the permissible limits (Table 1) Cr, Cu, Zn, and Cd and Fe were found to be 0.58 mg/l, 0.63 mg/l, 1.44 mg/l, and 0.107 mg/l respectively. Since the effluent is rich in heavy metals, the growth of *Vigna* is affected, which are irrigated by this effluent at different concentration (Table 5). These results of the physicochemical analysis coincide with earlier reports (Madan and Pertibha, 2013) [17]. Effluent loaded with heavy metals leads to increase in concentration of metals in soil and vegetation (Satpati *et al.*, 2010) [27]. Moreover, heavy metals accumulation have adverse impact on chlorophyll content on various vegetables, crops and alters the biomass production and yield (Sharma *et al.*, 2008, Medhi *et al.*, 2011) [30, 20]. But, at the same time, certain plants which accumulate heavy metals in greater quantities can serve as source of phytoremediation (Rai, 2010) [24].

Electrical conductivity is highly correlated with phosphate (r =0.968), Mg (r =0.954), Ca (r =0.908), while carbon is highly correlated with organic matter (r = 0.877) present in effluent. Mg & EC (r =0.954) were found to have high correlation value. Mg & Ca (r = 0.907) were highly correlated, while Na & Mg (r =0.454) were found to show lesser degree of correlation. Carbon was found to be negatively correlated with Nitrogen (r =-0.885). Nitrogen and Potassium showed lesser degree (r =0.044) degree of correlation. Narasimha *et al.*, (2011) [22] reported similar findings with cotton ginning mill effluent.

From Table 2, it is evident that pH of soil increased with increasing concentration due to presence of high amount of CO_3 and Ca in paper mill effluent. Madan and Pertibha, 2013) [17]. In present study, organic matter, Ca, Mg, K, Na concentration decreased with increasing the effluent concentration. Organic matter was found high in soils irrigated with 10% of effluent concentration. Sodium values were found highest in soils irrigated with 50 percent of effluent concentration. Tripathi *et al.*, (2014) [34] has revealed that soils irrigated swith paper mill effluent have high microbial diversity both in term of structure and function than the control soil.

Table 1: Mean value of different parameters (mg/l) after effluent which was used in different dilutions. Mean value along with Standard Deviation, n=5.

S. No.	Parameters	Average	SD
1.	pH	7.42	0.371
2.	EC(mmho / cm)	1506.2	58.486
3.	TDS	1311.4	30.342
4.	DO	1.26	0.242
5.	BOD	294.9	19.821
6.	COD	701.2	24.303
7.	Na	304.2	39.555
8.	K	378	67.814
9.	Chloride	647.8	87.303
10.	Alkalinity	702.6	28.204
11.	Nitrate	357.4	24.532
12.	TKN	487.6	151.550
13.	Salinity	0.68	0.147
14.	Turbidity	66.8	2.135
15.	Phosphate	710.6	100.039
16.	Cu	0.63	0.196
17.	Zn	1.44	0.963
18.	Pb	0.56	0
19.	Hg	0.00	0.000
20.	Cd	0.107	0.048
21.	Cr	0.58	0.341

At more than 10% effluent concentration, test plant showed inhibitory effect on growth on roots shoots length biomass. Similar type of tolerance studies were carried out on seven varieties of finger millet to factory effluent. The varietal screening of groundnut for tolerance to fertilizer factory effluent (Sundaramoorthy, 1995) [32] and sugar mill effluent (Ezhumalai 2002) [9], were reported. Similarly, the effect on paddy cultivars for tolerance to tannery effluent and Sugar mill effluent (Sundaramoorthy and Lakshmanachary, 2003) [33] was reported. High amount of chemicals and salt and high concentration of sodium increases the exchangeable sodium percentage of the soil to high level of phytotoxicity. The maximum growth of *V. mungo* was noted upto 10% of effluent. Coefficient of correlation analysis and regression analysis is given in figure one and two.

Compared to control, total chlorophyll content was found to be most sensitive parameter. Further the metals present in paper mill effluent generate active oxygen species and reduced total chlorophyll may be due to phytotoxic consequences of reactive oxygen species (Chandra *et al.*, 2010) [5]. Reduced protein content in above 20% effluent irrigated plants may also be attributed to the presence of toxic concentration of heavy metals in effluent. However, the increase in Protein content in *V. mungo* exposed to low concentration of paper mill effluent may be due to synthesis of stress protein. Bharagava *et al.*, (2008) [3] reported that distillery effluent irrigation increase chlorophyll and protein contents in Indian mustard plants (*Brassica nigra* L.) at 25 and 50% sugar mill effluent concentrations followed by decrease in 75% and 100% sugar mill effluent.

The study revealed that at more than 10% concentration of effluent all the growth parameters showed decline except protein content. The improvement of vegetative growth may be attributed to the role of potassium in nutrient and sugar translocation in plants and turgor pressure in plant cells. It is also involved in cell enlargement and in triggering young tissue or meristematic growth. (Kumar, *et al.* 2012) [15] Chlorophyll content was higher due to use of 20% dilution of paper mill effluent. This contains optimum contents of nutrients required for maximum vegetative growth of *Vigna mungo T-9.* This coincides with the results of Medhi *et al.*, (2011) [20], Madan and Pertibha (2013) [17] and Kumar *et* *al*., (2013) [14], Mishra *et al*. (2013) [21] regarding paper mill effluent. Along with this analysis of soil samples irrigated by different effluent concentration have shown that effluent increases the soil mineral content and organic matter in small quantities. From the above results it can be concluded that, effluent can be used safely for *V. mungo var T-9* cultivation only after proper dilution up to certain extent.

Table 2: Values of different soil parameters (mg/l)s at different concentrations of effluent

PME Conc. per 2kg	pH	EC (mmho / cm)	C%	Organic Matter	N%	PO_4 mg/l	Na	K	Mg	Ca
Before PME Application	6.2	0.345	0.42	0.81	0.08	0.8	21.7	3.6	11.7	75
10%	6.3	0.346	0.59	1.07	0.03	0.81	16.6	1.5	13.7	76
20%	6.5	0.355	0.52	0.89	0.03	0.97	17.5	2.2	14.1	77
30%	6.9	0.359	0.53	0.91	0.03	1.31	20.5	3.7	15.2	88
40%	7.3	0.378	0.57	0.93	0.02	1.77	22.5	4.7	16.4	95
50%	7.4	0.396	0.59	0.96	0.03	1.91	22.6	5.3	18.5	95

Table 3: Correlation among different parameters in soil analysis.

	pH	EC	C%	OM	N%	PO_4	Na	K	Mg	Ca
pH	1									
EC	0.947	1								
C%	0.583	0.56	1							
OM	0.155	0.152	0.877	1						
N%	-0.608	-0.488	-0.885	-0.665	1					
PO_4	0.993	0.968	0.547	0.124	-0.53	1				
Na	0.652	0.647	-0.142	-0.455	0.18	0.707	1			
K	0.819	0.824	0.081	-0.298	-0.044	0.86	0.961	1		
Mg	0.947	0.954	0.743	0.367	-0.694	0.938	0.454	0.675	1	
Ca	0.988	0.908	0.544	0.139	-0.558	0.983	0.706	0.845	0.907	1

Table 4: Values of chlorophyll and protein content (mg/gm) of *V. mungo* at different concentrations of Paper Mill Effluent

S. No.	Effluent	Chl mg/g	Protein
1.	0%	4.4 ± 0.22	18.46
2.	5%	4.7 ± 0.08	18.5 ± 0.5
3.	10%	4.9 ± 0.16	19.7 ±0.5
4.	20%	3.6 ± 0.205	23.5 ± 1.5
5.	30%	3.1 ± 0.04	18.7 ± 1.5
6.	40%	2.6 ± 0.13	12.2 ± 1.5
7.	50%	2.5 ± 0.1	9.1 ± 1.2

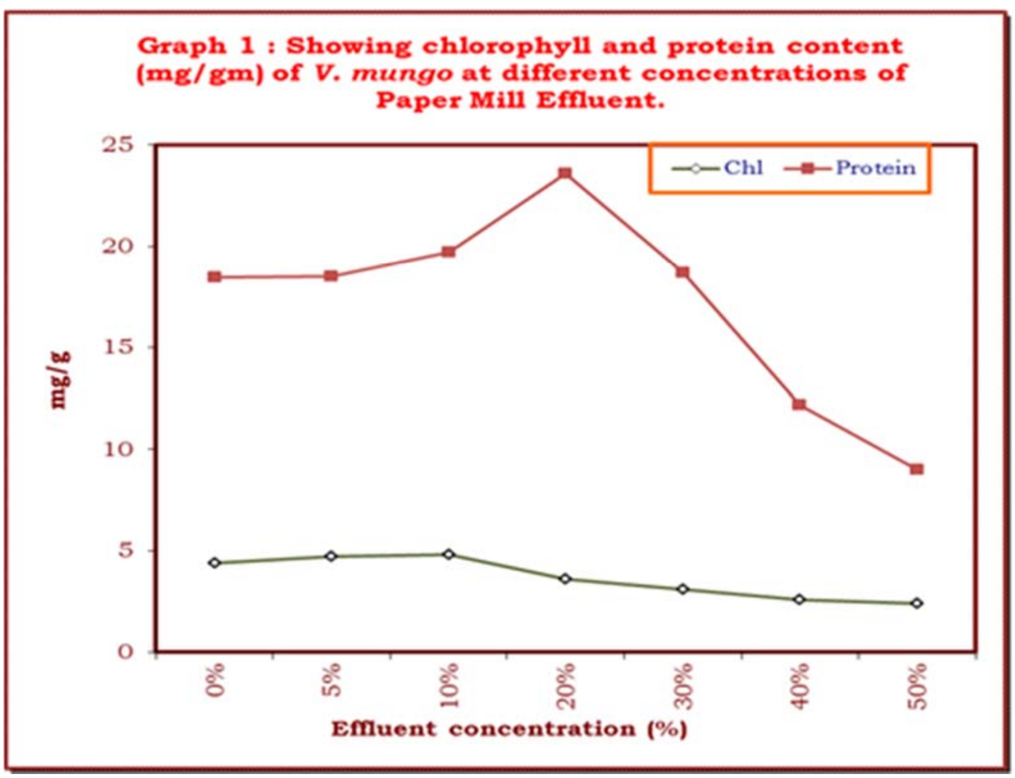
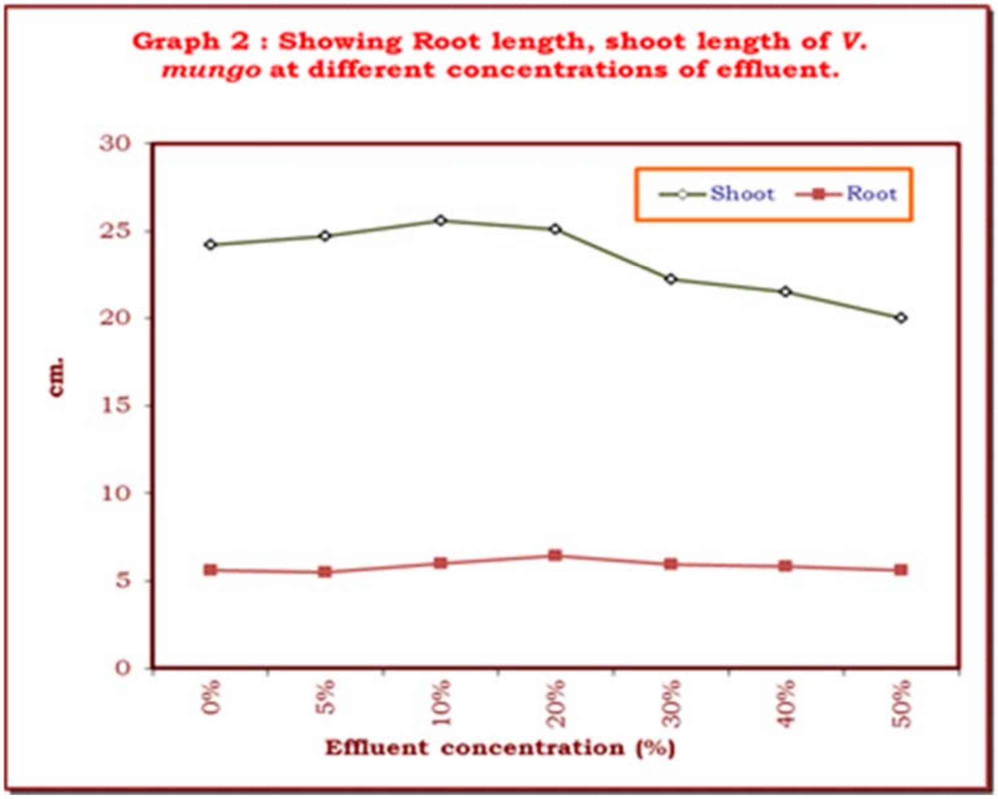


Table 5: Root length, shoot length and biomass of *V. mungo* at different concentrations of effluent

S. No.	Effluent concentration (%)	Shoot Length (cm.)	Root Length (cm.)	Biomass per plant (gm)
1.	0%	24.2±0.25	5.6±0.05	0.264±0.005
2.	5%	24.7± 0.25	5.5±0.4	0.283±0.003
3.	10%	25.6 ±0.4	6.00±0.1	0.317±0.007
4.	20%	25.1±0.85	6.4±0.05	0.258±0.0315
5.	30%	22.2±1.2	5.9±0.4	0.23±0.003
6.	40%	21.5±0.5	5.8±0.1	0.225±0.025
7.	50%	20±1	5.6±0.1	0.17±0.025



Thus it can be concluded that at lower concentration effluent increases the plant growth (Rana *et al.*, 2012 and Iqbal *et al.*, 2013) [25, 11] At more than 20% effluent concentration, test plant showed inhibitory effect on growth on roots shoots length biomass. Further the metals present in paper mill effluent generate active oxygen species and reduced total chlorophyll may be due to phytotoxic consequences of reactive oxygen species (Chandra *et al.*, 2010) [5].

Reduced protein content in above 20% effluent irrigated plants may also be attributed to the presence of toxic concentration of heavy metals in effluent. However, the increase in protein content in *V. mungo* exposed to low concentration of paper mill effluent may be due to synthesis of stress protein. Biochemical parameters, chlorophyll and protein declined clearly above twenty percent of effluent concentration which resulted in decrease in root length, shoot length leaf area and biomass of *Vigna mungo* T-9.

4. Conclusion

Since industrial effluent poses a ecological risk. Phytotoxic effects of paper mill effluent and ferti-irrigation studies of effluent with black gram and chlorophyll and protein analysis of it in black gram are highly valuable since *Vigna mungo* T-9 is prominent pulses of north India. Further, incorporation with one other remedial technique such as soil amendments, sprinkler irrigation, drip irrigation and intercropping system can reduce the phytotoxic effects of effluent and can improve ferti-irrigation potential of industrial effluent as shown by *Vigna mungo* T-9 at optimum level of dilution.

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