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Ferti-irrigational Effect of Paper Mill Effluent on Agronomical Characteristics of *Abelmoschus esculentus* L. (Okra)

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Abstract: The ferti-irrigational effect of an agro-based paper mill effluent on *Abelmoschus esculentus* (var. IHR-31) was investigated. Different doses of paper mill effluent viz. 5, 10, 25, 50, 75 and 100% were used for fertigation of *A. esculentus* along with bore well water (control). The study revealed that paper mill effluent had significant ($p<0.05$) effect on EC, pH, OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, TKN, PO_4^{3-}, SO_4^{2-}, Cd, Cr, Cu, Mn and Zn of the soil in both seasons. Insignificant ($p>0.05$) changes in WHC and bulk density of the soil were observed after irrigation with paper mill effluent. The agronomical performance of *A. esculentus* was increased from 5 to 25% and decreased from 50 to 100% concentration of paper mill effluent as compared to control in both seasons. The heavy metals concentration was increased in *A. esculentus* from 5 to 100% concentrations of paper mill effluent in both seasons. Biochemical components like crude proteins, crude fiber and crude carbohydrates were found maximum with 25% paper mill effluent in both seasons. The order of Contamination Factor (Cf) of various heavy metals was Cr>Cd>Mn>Zn>Cu for soil and Zn>Mn>Cu>Cr>Cd for *A. esculentus* plants after fertigation with paper mill effluent. Therefore, paper mill effluent can be used as a biofertigant after appropriate dilution to improve yield of *A. esculentus*.

Key words: *Abelmoschus esculentus*, agronomical characteristics, contamination factor, ferti-irrigation, heavy metals, paper mill effluent

INTRODUCTION

The value of wastewater for crop production has been recognized in arid and semi arid regions of the world including India (Feldkirchner *et al*., 2003; Calacea *et al*., 2005; Battaglia *et al*., 2007; Medhi *et al*., 2008; Kumar, 2010). Effluent is an important source of irrigation water as well as a source of plant nutrients such as Nitrogen (N), Phosphorus (P), Potassium (K) and trace elements like Sodium (Na), Calcium (Ca) and Magnesium (Mg) (Kumar and Chopra, 2012; Chopra and Pathak, 2013). Effluent irrigation can eliminate water shortage; reduce the need for chemical fertilizer and enhance the soil fertility (Srinivas *et al*., 1999; Dhevagi *et al*., 2000; Kumar, 2010). However, unregulated irrigation with untreated effluent poses serious public health risks, as effluent is a major source of heavy metals that cause accumulation in plant parts (Gomathi and Oblisami, 1992; Singh *et al*., 1996; Dutta and Boissya, 1998). The effluent contains heavy metals such as Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Nickel (Ni) and Zinc (Zn) which accumulates in plant and vegetable parts and cause adverse health effects (Hati *et al*., 2007; Chopra and Pathak, 2013). Long term irrigation with effluents increases organic carbon and heavy metals accumulation in soil and increase the chances of their entrance in food chain and this ultimately causes significant geoaccumulation, bioaccumulation and biomagnifications (Chopra *et al*., 2009; Srivastava *et al*., 2012).

The pulp and paper making industry is one of the major effluent generation industries in the world (Howe and Wagner, 1996; Dutta and Boissya, 1999; Chaudhary *et al*., 2002). In recent years, the use of treated, partially treated or raw effluent for irrigating productive agriculture or forest crops has become a popular alternative to discharge into surface water bodies (Kannan and Oblisami, 1990; Srivastava, 1991; Fazeli *et al*., 1998; Phukan and Bhattacharyya, 2003). Among agro-based industries, pulp and paper mills are one of the most polluting industries in India (Chaudhary *et al*., 2002). India has 666 pulp and paper mills, out of which 632 mills are agro-residue based mills (Kumar and Chopra, 2012). They generate a huge amount of wastewater (black liquor) having high Biological Oxygen Demand (BOD) and chemical oxygen demand (COD) values (Howe and Wagner, 1999; Kumar and Chopra, 2012; Pathak *et al*., 2013).

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Abelmoschus esculentus (L.) is an economically valuable vegetable crop grown in tropical and sub-tropical countries of the world including India (Chopra *et al*., 2012). This crop is suitable for cultivation as a garden crop as well as on large commercial farms. It is grown commercially in India, Pakistan, Bangladesh, Afghanistan, Turkey, Iran, Burma, Japan, Malayasia, Brazil, Ghana, Ethiopian and the Southern United States (Chopra *et al*., 2012; Pathak *et al*., 2012). Globally India ranks first with 3.5 million tonnes (70% of the total global yield) of *A. esculentus* produced from over 0.35 million ha land (Chopra *et al*., 2012).

Some crops have higher potential yields with effluent irrigation. It is an important to know the crop-effluent relationship for their suitable use in cultivation of agricultural crops (Kumar, 2010; Chopra and Pathak, 2012). Recently various investigations have been carried out on the use of industrial effluents in cultivation of various agricultural crops (Kannan and Oblisami, 1990; Singh *et al*., 2002; Hati *et al*., 2007; Kumar, 2010). But most studies were conducted on few agronomic stages with limited parameters in various crops, but there are few reports on comprehensive agronomic studies at various agronomic stages of these plants (Kaushik *et al*., 2005; Kumar, 2010). Thus, much attention has not been paid so far on the use of industrial effluents on the cultivation of agricultural crops like *A. esculentus*. Keeping in view the reuse of effluent as fertigant and the economic importance of *A. esculentus*, the present investigation was undertaken to study the ferti-irrigational effect of paper mill effluent on agronomical characteristics of *Abelmoschus esculentus* (L.).

MATERIALS AND METHODS

Experimental design: A field study was conducted at the Experimental Garden of the Department of Zoology and Environmental Sciences, Faculty of Life Sciences, Gurukula Kangri University Haridwar, India (29°55'10.81" N and 78°07'08.12" E), to study the ferti-irrigational effects of paper mill effluent on *A. esculentus*. The crop was cultivated in the summer and rainy seasons in 2010 and 2011. Six plots (each plot had an area of 9 m^2) were selected for six treatments of paper mill effluent viz. 0% (control), 10, 25, 50, 75 and 100% for the cultivation of *A. esculentus*. The six treatments were placed within each of the six blocks in a randomized complete block design.

Effluent collection and analysis: The effluent samples were provided from the Shamli Paper mill, Shamli (Uttar Pradesh), which produces paper from agricultural waste or residues. Effluent waste collected from a settling tank installed on the campus, by the paper mill, to reduce Biological Oxygen Demand (BOD) and solids from the paper mill in plastic container and were brought to the laboratory and analyzed for Total Dissolved Solids (TDS), pH, Electrical Conductivity (EC), Dissolved Oxygen (DO), BOD, COD, Chlorides (Cl^-), bicarbonates (HCO_3^-), carbonates (CO_3^{2-}), sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), Total Kjeldahl Nitrogen (TKN), nitrate (NO_3^{2-}), phosphate (PO_4^{3-}), sulphate (SO_4^{2-}), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), standard plate count (SPC) and most probable number (MPN) following standard methods (American Public Health Association, 2005; Chaturvedi and Sankar, 2006) and used as fertigant.

Soil preparation, filling of bags, sampling and analysis: The loamy soil was collected from a depth of 0-15 cm. Each bag (30×30 cm) was filled with 5 kg of soil which had been air-dried and sieved to remove debris and mixed with equal quantity of cow manure. The soil in each bag was fertigated twice in a month with 50 gallons of paper mill effluent with 5, 10, 25, 50, 75 and 100% along with well water as the control. The soil was analyzed prior to planting and after harvest for various physico-chemical parameters like soil texture, Bulk Density (BD), Water Holding Capacity (WHC), EC, pH, OC, Na^+, K^+, Ca^{2+}, Fe^{2+}, Mg^{2+}, PO_4^{3-}, SO_4^{2-}, TKN, Cd, Cr, Cu, Mn and Zn determined following standard methods cited by Chaturvedi and Sankar (2006).

Sowing of seeds and irrigation pattern: Seed of *A. esculentus* were sown at the end of February 2010 and 2011 for the summer crop and at the end of July 2010 and 2011 for the rainy season crop. Seed of *A. esculentus*, cv. IHR-31, were procured from ICAR, Pusa, New Delhi and sterilized with 0.01% mercuric chloride and soaked in water for 12 h. Seeds were sown in 10 rows with a distance of 30.0 cm between rows, while distance between the seeds was 15 cm. The thinning was done manually after 15 days of germination to maintain the desired plant spacing and to avoid competition between plants. The plants in each plot were fertigated twice in a month with 50 gallons of paper mill effluent with 5, 10, 25, 50, 75 and 100% along with bore well water as the control.

Study of crop parameters: The agronomic parameters of *A. esculentus* at different stages (0-90 days) were determined following standard methods for seed germination, plant height, root length, number of flowers, number of fruits, fruits length and crop yield (Chandrasekar *et al*., 1998); dry weight (Milner and Hughes, 1968); chlorophyll content (Porra, 2005); Relative

Toxicity (RT) (Chapagain, 1991), Leaf Area Index (LAI) (Denison and Russotti, 1997) and harvest index (HI) (Sinclair, 1998). The nutrient quality of *A. esculentus* was determined by using the following parameters; crude protein (4.204 AOAC, 1980), crude fiber (4.601 AOAC, 1980) and the total carbohydrate in dry matter were determined by the anthrone reagent method (Cerning and Guilbot, 1973).

Extraction of metals and their analysis: For metal analysis a 5-10 mL sample of paper mill effluent and 0.5-1.0 g of air dried soil or plants were digested in tubes with 3 mL of conc. HNO_3 digested in an electrically heated block for 1 h at 145°C. To this mix 4 mL of $HClO_4$ was added and heated to 240°C for 1 h. The mix was cooled and filtered through Whatman No. 42 filter paper and made to 50 mL and used for analysis. Metals were analyzed using an Atomic absorption spectrophotometer (PerkinElmer, Analyst 800 AAS, GenTech Scientific Inc., Arcade, NY) following methods of American Public Health Association (2005) and Chaturvedi and Sankar (2006). The Contamination factor (Cf) for metals accumulated in paper mill effluent irrigated soil and *A. esculentus* was calculated following Hakanson (1980).

Data analysis: Data were analyzed with SPSS (ver. 14.0, SPSS Inc., Chicago, Ill.). Data were subjected to one-way ANOVA. Mean standard deviation and coefficient of correlation (r-value) of soil and crop parameters with effluent concentrations were calculated with MS Excel (ver. 2003, Microsoft Redmond Campus, Redmond, WA) and graphs produced with Sigma plot (ver. 12.3, Systat Software, Inc., Chicago, IL).

RESULTS AND DISCUSSION

Characteristics of paper mill effluent: Values of physico-chemical and microbiological parameters varied over paper mill effluent concentration (Table 1). The paper mill effluent was alkaline i.e., pH 8.76. The alkaline nature of the paper mill effluent might be due to presence of high concentrations of alkalis used in pulping. The BOD, COD, Cl^-, Ca^{2+}, Fe^{2+}, TKN, SO_4^{2-}, MPN and SPC were above the prescribed limits of the Indian Irrigation Standards (BIS, 1991). High BOD and COD might be due to presence of high utilizable organic matter and rapid consumption of dissolved inorganic materials. The higher bacterial load (SPC and MPN) in paper mill effluent might be due to presence of more dissolved solids and organic matter in effluent as earlier reported by Kumar (2010). The TKN, PO_4^{3-}, K^+, Ca^{2+} and Mg^{2+} in effluent were higher than the prescribed standards (Table 1).

In the present study, the content of BOD, COD, TKN, Cl^-, SO_4^{2-} and PO_4^{3-} were more in paper mill effluent then the content of BOD (635.78 mg L^{-1}), COD (964.56 mg L^{-1}), total nitrogen (36.45 mg L^{-1}), chlorides (324.48 mg L^{-1}), sulphate (468.50 mg L^{-1}) and phosphate (46.75 mg L^{-1}) in paper mill effluent reported by Singh *et al.* (2002). In the case of metals, the contents of Cd, Cr Cu, Fe, Mn and Zn were higher than permissible limits for industrial effluent (BIS, 1991). The content of these metals in paper mill effluent were also higher then the content of Cd (11.42 mg L^{-1}), Cr (12.88 mg L^{-1}) Cu (8.65 mg L^{-1}) and Zn (16.44 mg L^{-1}), in paper mill effluent reported by Thompson *et al.* (2001).

Effect of paper mill effluent on characteristics of soil: Physico-chemical characteristics of the soil characteristics changed due to irrigation with paper mill effluent. At harvest (90 days after sowing) there was no significant change in the soil texture (loamy; 40% sand: 40% silt: 20% clay). WHC and BD were insignificantly ($p>0.05$) affected by different concentrations of paper mill effluent in both the cultivated seasons (Table 2). Season, paper mill effluent concentration and the their interaction affected OC, TKN, all cations like Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, anions PO_4^{3-} and SO_4^{2-} and metals Cd, Cr, Cu, Mn and Zn of the soil (Table 2-4). It has also been observed that effluent irrigation generally adds OC, Na^+, Ca^{2+}, K^+, Mg^{2+}, PO_4^{3-}, HCO_3^-, Cl^- Zn, Cd, Cr, Cu, Ni and Mn to the soil (Pokhrel and Viraraghavan, 2004; Patterson *et al*., 2008; Chopra *et al*., 2009). WHC and BD were reduced from their initial (control) values 42.86% and 1.40 g cm^{-3} to 41.15% and 1.39 g cm^{-3}, respectively with 100% paper mill effluent concentration. The pH of the soil was turned alkaline to more alkaline (8.86 and 8.96) after irrigation with 100% paper mill effluent in both seasons (Table 5). The change in soil pH and reduction in WHC and BD after paper mill effluent irrigation have also been observed earlier by Kumar and Chopra (2012).

Paper mill effluent had significant ($p<0.01$) effect on EC, pH, OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, TKN, PO_4^{3-}, SO_4^{2-}, Fe, Zn, Cu, and Mn of the soil in both seasons (Table 5).

In the present study, more irrigation of *A. esculentus* considerably increased the content of OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, TKN, PO_4^{3-}, SO_4^{2-} Zn, Cd, Cu, Mn and Cr in soil. Soil pH was affected by the 50, 75 and 100% paper mill effluent concentrations (Table 5). The 25 to 100% paper mill effluent concentrations significantly ($p<0.05$) affected EC, OC, TKN, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, PO_4^{3-}, SO_4^{2}, Cu, Cr, Cd, Mn and Zn in *A. esculentus* cultivated soil in both seasons (Table 5, 6). Irrigation with 100% paper mill effluent had the most reduction in WHC, BD; and increase in EC, OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, TKN,

Table 1: Physico-chemical and microbiological characteristics of paper mill effluent

	Effluent concentration (%)							BIS for irrigation water
Parameter	0 (BWW)	5	10	25	50	75	100	
TDS (mg L^{-1})	221.5	1420.00	1864.00	2386.00	2675.00	2967.00	4064.00	1900
EC (dS m^{-1})	0.3	2.34	2.86	3.96	4.12	4.98	6.56	-
pH	7.5	7.63	7.71	7.88	7.99	8.25	8.76	5.5-9.0
DO (mg L^{-1})	8.24	5.26	4.17	3.42	2.31	1.62	NIL	-
BOD (mg L^{-1}	3.8	75.69	132.75	314.76	624.88	944.86	1236.96	100
COD (mg L^{-1})	5.9	162.47	312.00	832.66	1542.82	2245.75	2963.88	250
Cl^{-} (mg L^{-1})	15.7	74.88	125.64	235.00	358.75	668.80	875.98	500
HCO_3^- (mg L^{-1}	282.0	276.84	286.66	310.40	384.75	436.50	568.82	-
CO_3^{2-} (mg L^{-1})	105.8	124.45	152.60	176.48	186.70	214.50	236.60	-
Na^+ (mg L^{-1})	9.7	35.76	74.68	136.86	264.54	376.83	498.30	-
K^+ (mg L^{-1})	5.5	22.74	45.36	96.86	176.69	234.85	264.77	-
Ca^{2+} (mg L^{-1}	23.5	65.30	121.15	246.75	436.55	576.84	789.64	200
Mg^{2+} (mg L^{-1})	12.2	21.44	45.86	86.96	176.69	246.86	312.58	-
TKN (mg L^{-1})	24.3	35.12	72.86	96.26	146.75	175.36	243.86	100
NO_3^{2-} (mg L^{-1})	25.2	52.44	112.36	167.45	236.87	312.44	396.86	100
PO_4^{3-} (mg L^{-1})	0.04	9.26	20.45	64.75	123.47	186.60	235.96	-
SO_4^{2-} (mg L^{-1})	17.6	54.75	98.36	186.48	348.60	486.96	66.84	1000
Fe^{2+} (mg L^{-1})	0.3	1.12	2.26	4.68	9.68	14.86	19.36	1.0
Cd (mg L^{-1})	0.06	0.56	1.12	2.43	4.46	6.86	9.98	15
Cr (mg L^{-1})	0.1	1.42	2.96	5.26	8.76	12.48	17.86	2.00
Cu (mg L^{-1})	0.04	1.16	2.38	5.48	8.67	11.42	16.36	3.00
Mn (mg L^{-1})	0.02	0.76	1.52	4.26	7.75	10.64	14.83	1.00
Zn (mg L^{-1})	0.04	0.66	1.13	3.25	6.75	8.36	12.47	2.00
SPC (SPC mL^{-1})	63	5.4×10^5	7.9×10^6	6.7×10^7	5.8×10^8	6.6×10^9	8.6×10^{12}	10000
MPN (MPN100 mL^{-1})	2.6×10^1	3.6×10^4	8.3×10^5	7.9×10^6	6.4×10^7	5.9×10^8	7.6×10^{10}	5000

BWW: Bore well water. BIS: Bureau of Indian standard, Least squares means analysis

Table 2: ANOVA for effect of paper mill effluent on soil characteristics

Source	WHC	BD	EC	pH	OC	TKN
Season (S)	ns	ns	ns	ns	*	*
PME concentration (C)	ns	ns	**	*	**	**
Interaction						
S × C	ns	ns	*	*	**	**

ns, *,**non-significant or significant at p<0.05 or p<0.01, ANOVA

Table 3: ANOVA for effect of paper mill effluent on concentrations of cations and anions

Source	Na^+	K^+	Ca^{2+}	Mg^{2+}	Fe^{2+}	PO_4^{3-}	SO_4^{2-}
Season (S)	*	*	*	*	*	*	*
PME concentration (C)	**	*	*	*	**	**	**
Interaction							
S × C	**	**	**	**	**	**	**

*, **Significant at p<0.05 or p<0.01, ANOVA

Table 4: ANOVA for effect of paper mill effluent on concentrations of metals

Source	Cd	Cr	Cu	Mn	Zn
Season (S)	*	*	*	ns	*
PME concentration (C)	**	**	**	*	**
Interaction					
S × C	**	**	**	**	**

*,**Significant at p<0.05 or p<0.01, ANOVA

PO_4^{3-}, SO_4^{2-}, Cd, Cr, Cu, Mn and Zn in both seasons (Table 5, 6). The findings were very much in accordance with Phukan and Bhattacharyya (2003).

Total average organic matter content in the soil irrigated with effluent was higher than the soil irrigated with bore well water. The more organic matter in effluent irrigated soil might be due to the high organic nature of the effluent. Kumar and Chopra (2012) found the organic content in the soil irrigated with paper mill effluent to be higher than in the soil irrigated with bore well water. Average values of TKN, PO_4^{3-} and K^+ in the soil irrigated with effluent were found to be higher than in soil irrigated with bore well water. The high amount of TKN, PO_4^{3-} and K^+ in the soil was due to irrigation with TKN, PO_4^{3-} and K^+ rich paper mill effluent. The content of Na^+ and SO_4^{2-} was higher in the soil irrigated with paper mill effluent indicating a link between soil Na^+ and SO_4^{2-} and higher EC in the paper mill effluent.

The soil parameters, EC, OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, TKN, PO_4^{3-}, SO_4^{2-} Zn, Cd, Cu, Mn and Cr positively correlated with paper mill effluent concentration in both seasons (Table 7). The Contamination factor (Cf) of the metals indicated that Cr was highest while Cu was lowest in both seasons after irrigation with 100% paper mill effluent. The Cf of metals were in the order of Cr>Cd>Mn>Zn>Cu after irrigation with paper mill effluent in both seasons (Fig. 1). The concentrations of metals were higher in soil irrigated with effluent than in soil irrigated with control water. Thus, fertigation with distillery effluent increased nutrients as well as metals content in soil. Enrichment of various metals was also observed by Fazeli *et al.* (1998) in soil after paper mill effluent irrigation.

Effect of paper mill effluent on seed germination of *A. esculentus*: At 0-15 days after sowing, the best germination (97 and 94%) was for with control and the

Table 5: Effects of paper mill effluent concentration and season interaction on physico-chemical characteristics of a loamy soil before and after irrigation of *A. esculentus* in both seasons

Season × %PME	EC (dS m^{-1})	pH	OC (mg kg^{-1})	Na^+ (mg kg^{-1})	K^+ (mg kg^{-1})	Ca^{2+} (mg kg^{-1})	Mg^{2+} (mg kg^{-1})
Rainy							
0	2.12	7.66	0.48	18.56	162.33	16.48	1.74
5	2.56ns	8.13ns	1.68*	26.36 ns	175.39 ns	36.46 ns	4.23 ns
10	2.67ns	8.28ns	3.24*	28.71*	184.69 ns	42.58 ns	7.12 ns
25	2.88*	8.39ns	4.68**	32.99*	196.86*	82.75*	10.42*
50	2.98*	8.54*	5.76**	38.96*	218.73*	123.46*	13.76*
75	3.13*	8.79*	7.86**	42.75**	234.40**	144.60*	16.86*
100	3.21**	8.86*	9.45**	48.39**	239.86**	168.96**	24.75*
Summer							
0	2.15	7.67	0.49	18.64	164.54	16.88	1.78
5	2.72ns	8.19ns	1.94*	28.69 ns	180.40 ns	39.80 ns	4.36 ns
10	2.88ns	8.34ns	4.76*	30.74*	204.66 ns	47.64 ns	7.63 ns
25	2.96*	8.45ns	6.35**	35.69*	212.64*	89.74*	11.24*
50	3.11*	8.66*	8.88**	40.58*	225.48*	132.45*	15.74*
75	3.21*	8.85*	9.12**	46.86**	237.46**	158.75*	18.36*
100	3.29**	8.96*	10.24**	52.48**	243.50**	176.42**	26.73*ns,

*,**non-significant or significant at $p<0.05$ or $p<0.01$, Least squares means analysis

Table 6: Effects of paper mill effluent concentration and season interaction on physico-chemical characteristics of a loamy soil before and after irrigation of *A. esculentus* in both seasons

Season × %PME	TKN (mg kg^{-1})	PO_4^{3-} (mg kg^{-1})	SO_4^{2-} (mg kg^{-1})	Fe^{2+} (mg kg^{-1})	Cd (mg kg^{-1})	Cr (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})
Rainy									
0	32.45	52.75	75.96	2.75	0.76	0.84	2.06	0.43	1.02
5	58.86 ns	68.96 ns	79.86 ns	3.36*	1.46*	1.62*	3.87*	1.12*	1.88*
10	66.48**	79.36 ns	86.76 ns	3.84*	2.75*	2.19*	3.94*	1.24*	2.42*
25	129.36**	98.94*	94.57*	5.76**	3.25**	3.76**	5.70**	1.76**	2.76**
50	186.55**	112.47**	109.34*	6.43**	3.86**	4.96**	5.99**	1.97**	2.94**
75	252.74**	124.32**	118.68**	7.64**	4.15**	5.87**	6.62**	2.14**	3.42**
100	296.86**	134.69**	129.37**	8.37**	5.42**	6.98**	8.90**	2.44**	4.75**
Summer									
0	33.46	53.14	76.42	2.82	0.78	0.88	2.08	0.45	1.04
5	62.44ns	74.55 ns	81.47 ns	3.57*	2.10*	1.81*	3.94*	1.19*	1.94*
10	74.64**	86.78*	88.79*	4.23*	2.98*	2.56*	4.13*	1.70*	2.49*
25	143.50**	102.16*	96.86*	5.96**	3.46**	4.53**	5.97**	1.86**	2.84**
50	196.69**	118.75**	117.67*	6.75**	3.98**	5.72**	6.78**	2.04**	3.02**
75	268.77**	129.86**	130.44**	8.24**	4.88**	6.83**	7.64**	2.24**	3.59**
100	302.44**	139.89**	141.37**	9.12**	5.76**	7.96**	9.67**	2.64**	4.86**

ns,*** non-significant or significant at $P<0.01$; Least squares means analysis

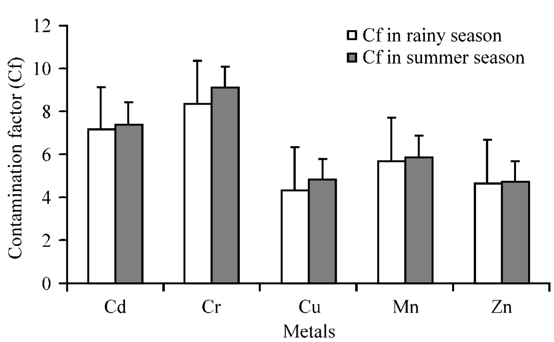

Fig. 1: Contamination factor of the metals in soil after irrigation with paper mill effluent. Error bars are standard error of the mean

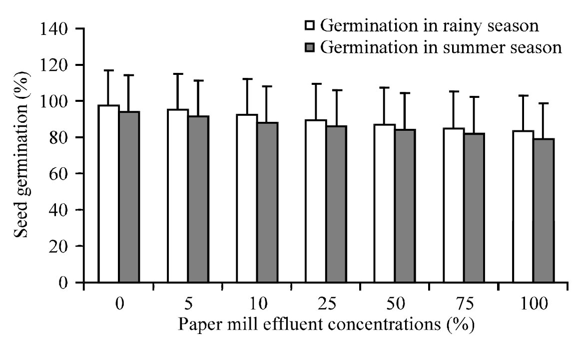

Fig. 2: Seed germination of *A. esculentus* after irrigation with paper mill effluent. Error bars are standard error of the mean

least (83 and 79%) was due to treatment with 100% paper mill effluent (Fig. 2). Germination was negatively correlated (r = -0.95) with paper mill effluent concentrations in both seasons. The ANOVA indicated that season had no significant ($p>0.05$) effect on seed germination and relative toxicity. Paper mill effluent

Table 7: Coefficient of correlation (r) between paper mill effluent and soil characteristics in both seasons

Paper mill effluent /soil characteristics	Season	r - value
Paper mill effluent versus soil WHC	Rainy	-0.95
	Summer	-0.96
Paper mill effluent versus soil BD	Rainy	-0.95
	Summer	-0.96
Paper mill effluent versus soil EC	Rainy	+0.87
	Summer	+0.81
Paper mill effluent versus soil pH	Rainy	+0.90
	Summer	+0.89
Paper mill effluent versus soil OC	Rainy	+0.97
	Summer	+0.91
Paper mill effluent versus soil Na^+	Rainy	+0.95
	Summer	+0.96
Paper mill effluent versus soil K^+	Rainy	+0.96
	Summer	+0.97
Paper mill effluent versus soil Ca^{2+}	Rainy	+0.91
	Summer	+0.97
Paper mill effluent versus soil Mg^{2+}	Rainy	+0.98
	Summer	+0.98
Paper mill effluent versus soil TKN	Rainy	+0.99
	Summer	+0.98
Paper mill effluent versus soil PO_4^{3-}	Rainy	+0.95
	Summer	+0.94
Paper mill effluent versus soil SO_4^{2-}	Rainy	+0.99
	Summer	+0.99
Paper mill effluent versus soil Fe^{2+}	Rainy	+0.97
	Summer	+0.97
Paper mill effluent versus soil Cd	Rainy	+0.92
	Summer	+0.93
Paper mill effluent versus soil Cr	Rainy	+0.97
	Summer	+0.97
Paper mill effluent versus soil Cu	Rainy	+0.94
	Summer	+0.96
Paper mill effluent versus soil Mn	Rainy	+0.91
	Summer	+0.87
Paper mill effluent versus soil Zn	Rainy	+0.82
	Summer	+0.81

concentration and their interaction with season affected seed germination of *A. esculentus*, but not relative toxicity (Table 8).

The maximum relative toxicity (116.86 and 118.98%) of paper mill effluent against germination was for the 100% paper mill effluent (Fig. 3) and it was positively correlated ($r = +0.54$) with paper mill effluent concentrations in both seasons. The findings are very much in accordance with Malla and Mohanty (2005) reported that the germination of green gram (*Phaseolus anreus* L.) was decreased as concentration of the paper mill effluent increased from 0 to 100%. The findings were also supported by Reddy and Borse (2001).

In the present investigation, the higher concentration of paper mill effluent did not support seed germination. The higher concentration of paper mill effluent lowered germination of *A. esculentus* likely due to presence of high salt content in the effluent at these concentrations. Seed take up water during germination and hydrolyse stored food material and to activate enzymatic systems. During germination salts can inhibit seed germination. The mechanism of inhibition of seed germination by NaCl

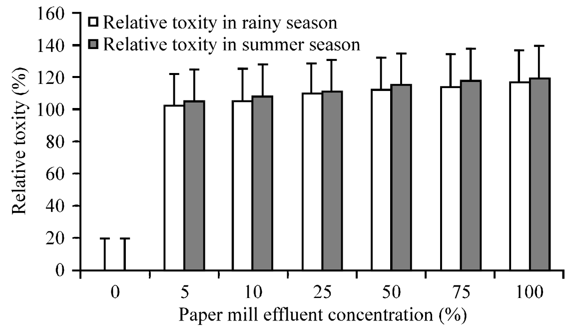

Fig. 3: Relative toxicity of paper mill effluent against seed germination of *A. esculentus*. Error bars are standard error of the mean

may be related to radicle emergence due to insufficient water absorption, or to toxic effects on the embryo. Seed that absorb an insufficient amount of water can accumulated a large amount of Cl^- when the osmotic pressure of the substrate is increased by salt concentration and as a result, the seeds emerged slowly, and at higher concentrations do not germinate (Patterson *et al*., 2008). High concentrations are usually most damaging to young plants but not necessarily at germination, although, high salt concentration can slow germination by several days, or completely inhibit it. Because soluble salts move readily with water, evaporation moves salts to the soil surface where they accumulate and harden the soil surface delays germination (Thompson *et al*., 2001; Kaushik *et al*., 2005).

Effect of paper mill effluent on vegetative growth of *A. esculentus*: Vegetative growth at 45 days was affected in both seasons (Table 8). Average plant height (112.45 and 116.64 cm), root length (16.85 and 19.36 cm), dry weight (74.63 and 76.87 g), chlorophyll content (3.36 and 3.48 mg./g.f.wt) and LAI/plant (3.64 and 3.68) of *A. esculentus* were observed with control while plant height (126.32 and 132.44 cm), root length (18.40 and 21.46 cm), dry weight (82.74 and 88.96 g), chlorophyll content (3.86 and 3.95 mg./g.f.wt) and LAI/plant (3.74 and 3.82) of *A. esculentus* were noted with 100% paper mill effluent in both seasons.

Maximum plant height (152.48 and 162.83 cm), root length (22.87 and 25.49 cm), dry weight (98.75 and 103.44 g), chlorophyll content (4.64 and 5.32 mg./g.f.wt) and LAI/plant (4.96 and 5.19) of *A. esculentus* were due to treatment with the 25% concentration of paper mill effluent in both seasons. The findings were also supported by Kamalakar *et al*. (1991). The ANOVA indicated that paper mill effluent concentration significantly ($p<0.05$) affected plant height and

Table 8: ANOVA for effect of paper mill effluent on germination and vegetative growth of *A. esculentus*

Source	Seed germination	Relative toxicity	Plant height	Root length	Dry weight	Chlorophyll content	LAI
Season (S)	ns	ns	ns	ns	ns	ns	ns
PME concentration (C)	*	ns	*	ns	ns	*	ns
Interaction (S×C)	*	ns	*	ns	ns	*	ns

ns, *,non-significant or significant at $p<0.05$, ANOVA

Table 9: Coefficient of correlation (r) between paper mill effluent and *A. esculentus* in both seasons

Paper mill effluent/ *A. esculentus*	Season	r-value
Paper mill effluent versus shoot length	Rainy	+0.66
	Summer	+0.65
Paper mill effluent versus root length	Rainy	+0.13
	Summer	-0.14
Paper mill effluent versus dry weight	Rainy	+0.23
	Summer	+0.38
Paper mill effluent versus chlorophyll content	Rainy	+0.27
	Summer	+0.27
Paper mill effluent versus LAI	Rainy	+0.47
	Summer	+0.50
Paper mill effluent versus no. of flowers/plant	Rainy	+0.59
	Summer	+0.27
Paper mill effluent versus soil no. of fruits	Rainy	+0.58
	Summer	+0.41
Paper mill effluent versus fruit length	Rainy	+0.48
	Summer	+0.32
Paper mill effluent versus crop yield/plant	Rainy	+0.10
	Summer	-0.06
Paper mill effluent versus harvest index	Rainy	+0.47
	Summer	+0.01
Paper mill effluent versus Cd	Rainy	+0.99
	Summer	+0.99
Paper mill effluent versus Cr	Rainy	+0.95
	Summer	+0.96
Paper mill effluent versus Cu	Rainy	+0.99
	Summer	+0.99
Paper mill effluent versus Mn	Rainy	+0.98
	Summer	+0.98
Paper mill effluent versus Zn	Rainy	+0.99
	Summer	+0.99

chlorophyll content of *A. esculentus* (Table 8). Season had no effect on plant height, root length, dry weight and LAI of *A. esculentus*. The interaction of season and paper mill effluent concentrations only affected plant height and chlorophyll content of *A. esculentus* (Table 8).

Plant height, dry weight, chlorophyll content and LAI/plant of *A. esculentus* were positively correlated with paper mill effluent concentrations in both seasons (Table 9). Root length was positively correlated with paper mill effluent concentrations in rainy season while it was negatively correlated in summer season (Table 9). Chopra *et al*. (2012) reported the maximum chlorophyll content in *A. esculentus* at 25% concentration of distillery effluent. Malla and Mohanty (2005) reported that paper mill effluent irrigation increase chlorophyll and protein contents in Indian mustard plants (*Phaseolus anreus* L.) at the 25 and 50% paper mill effluent concentrations followed by a decrease at 75 and 100% paper mill effluent. The findings were also supported by Sharma and Agrawal (2010) who reported that the growth of *A. esculentus* (L.) decreased when concentration of paper mill increased.

Vegetative growth of *A. esculentus* was decreased at higher concentrations of paper mill effluent. It is likely due to that higher salt content in the higher paper mill effluent concentrations, which lowered the plant height, root length, dry weight, chlorophyll content and LAI/plant of *A. esculentus*. Vegetative growth is associated with development of new shoots, twigs, leaves and leaf area. Plant height, root length, dry weight and LAI/plant of *A. esculentus* were higher at 25% of paper mill effluent it may be due to maximum uptake of nitrogen, phosphorus and potassium by plants. 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 mersitematic growth (Thompson *et al*., 2001). Chlorophyll content was higher due to use of 25% paper mill effluent in both seasons and is likely due to Fe, Mg and Mn contents in the paper mill effluent, which are associated w ith chlorophyll synthesis Porra (2005). The 25% paper mill effluent concentration contains optimum contents of nutrients required for maximum vegetative growth of *A. esculentus*.

Effect of paper mill effluent on flowering of *A. esculentus*: Numbers of flowers decreased as paper mill effluent concentration decreased (Table 9). At flowering stage (60 days after sowing) the maximum flowers (36.00 and 38.00) was noted with 25% paper mill effluent in both seasons. Numbers of flowers/plant 26.00 and 28.00 were with control and 30.00 to 32.00 with 100% paper mill effluent in both seasons. Season, paper mill effluent concentration and interaction of season and paper mill effluent concentration had no significant ($p>0.05$) effect on number of flowers and number of fruits/plant (Table 10).

Nitrogen and phosphorus are essential for flowering. Too much nitrogen can delay, or prevent, flowering while phosphorus deficiency is sometimes associated with poor flower production, or flower abortion. Maximum flowering was with the 25% paper mill effluent; it might be due to that this concentration contains sufficient nitrogen and phosphorus. Furthermore, P and K prevent flower abortion so pod formation occurs (El-Naggar, 2005). Flowering of *A. esculentus* was lower at higher concentrations of paper mill effluent. This is likely due to increased content of metals in the soil which inhibits uptake of P and K by plants at higher paper mill effluent concentrations (Pandey *et al*., 2008).

Effect of paper mill effluent on maturity of *A. esculentus*: The most fruits/plants (34.00 and 36.00), fruit length (9.75 cm and 10.38 cm) fresh yield/plant (760.54 and 775.86 g) and HI (750.05 and 770.16%) of *A. esculentus* were with the 25% paper mill effluent in both seasons. Numbers of fruits/plant, crop yield/plant and Harvest Index (HI) of *A. esculentus* were positively correlated with paper mill effluent concentrations in both seasons (Table 9). Numbers of fruits/plant, crop yield/plant and Harvest Index (HI) of *A. esculentus* were not affected by season, paper mill effluent concentration and their interaction (Table 10). The number of fruits/plants (23.00 and 26.00), fruit length (7.32 cm and 7.88 cm) fresh yield/plant (458.48 and 467.86 g) and HI (607.51 and 614.33%) of *A. esculentus* were with the control while with 100% paper mill effluent the fruits/plants (28.00 and 30.00), fruit length (8.78 cm and 8.96 cm) fresh yield/plant (568.84 and 584.74 g) and HI (657.30 and 687.50%) of *A. esculentus* were in both seasons.

The role of K, Fe, Mg and Mn at maturity is important and associated with synthesis of chlorophyll and enhances formation of fruits at harvest (El-Naggar, 2005; Naeem *et al*., 2006). The K, Fe, Mg and Mn contents could benefit pod formation and yield of as it does for soybean (*Glycine max* L.) as reported by Hati *et al*. (2007). The 25% paper mill effluent favored fruits formation and crop yield of *A. esculentus*. This is likely due to presence of K, Fe, Mg and Mn contents in 25% paper mill effluent; higher paper mill effluent concentrations lowered fruits formation and crop yield of *A. esculentus*.

Effect on biochemical constituents and micronutrients in *A. esculentus*: The content of various micronutrients were positively correlated with concentrations of paper mill effluent in both seasons (Table 9). Season, paper mill effluent concentration and the interaction of season and paper mill effluent concentration affected all the biochemical constituents like crude fiber and crude carbohydrates, and metals like Cd, Cr, Cu, Mn and Zn in *A. esculentus* (Table 11). Maximum crude proteins, crude fiber and crude carbohydrates were recorded with 25% paper mill effluent concentrations in both seasons (Fig. 4-6). Content of crude proteins ($r = +0.08$ and $r = +0.18$), crude fiber ($r = +0.14$ and $r = +09$) and crude carbohydrates ($r = +0.03$ and $r = +0.07$) were noted positively correlated with paper mill effluent concentration in both seasons. The 25, 50, 75 and 100% paper mill effluent concentrations affected Cd, Cr, Cu, Mn and Zn contents in *A. esculentus*. Increased irrigation frequency

Table 10: ANOVA for effect of paper mill effluent on flowering and maturity stage of *A. esculentus*

Source	No. of flowers/plant	No. of fruits	Fruit length	Crop yield/plant	Harvest index (HI)
Season (S)	ns	ns	ns	ns	ns
PME concentration (C)	ns	ns	ns	ns	ns
Interaction					
S × C	ns	ns	ns	ns	ns

ns: non-significant

Table 11: ANOVA for effect of paper mill effluent on concentrations of metals in *A. esculentus*

Source	Cd	Cr	Cu	Mn	Zn	Crude proteins	Crude fiber	Crude carbohydrates
Season (S)	*	*	*	ns	*	*	*	*
PME concentration (C)	**	**	**	**	**	**	**	**
Interaction								
S × C	**	**	**	**	**	**	**	**

ns,*,**non-significant or significant at $p<0.05$ or $p<0.01$, ANOVA

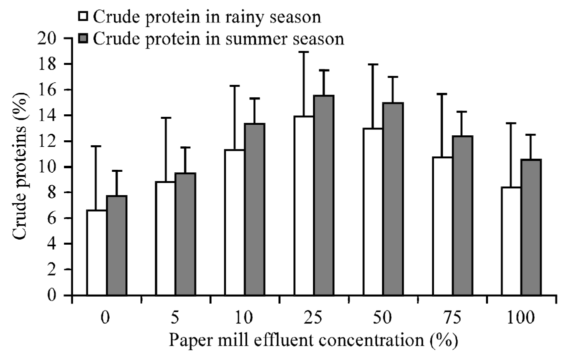

Fig. 4: Crude proteins in *A. esculentus* after irrigation with paper mill effluent. Error bars are standard error of the mean

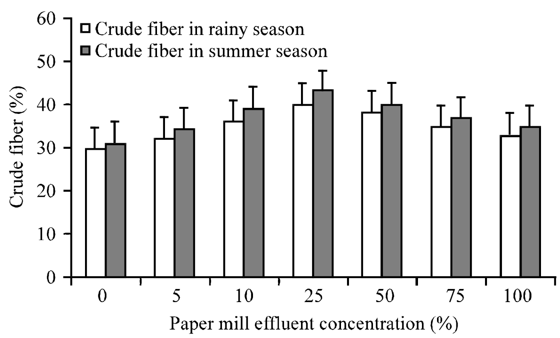

Fig. 5: Crude fiber in *A. esculentus* after irrigation with paper mill effluent. Error bars are standard error of the mean

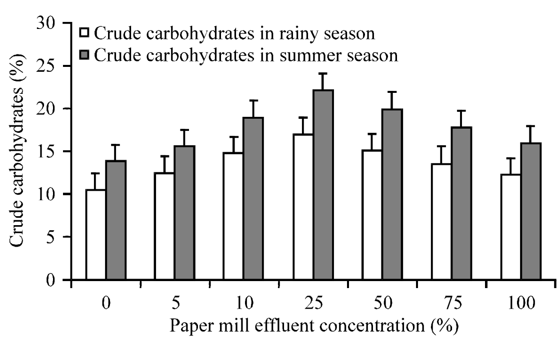

Fig. 6: Crude carbohydrates in *A. esculentus* after irrigation with paper mill effluent. Error bars are standard error of the mean

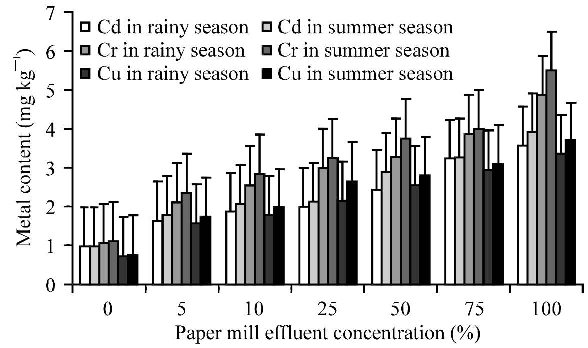

Fig. 7: Content of Cd, Cr and Cu in *A. esculentus* after irrigation with paper mill effluent. Error bars are standard error of the mean

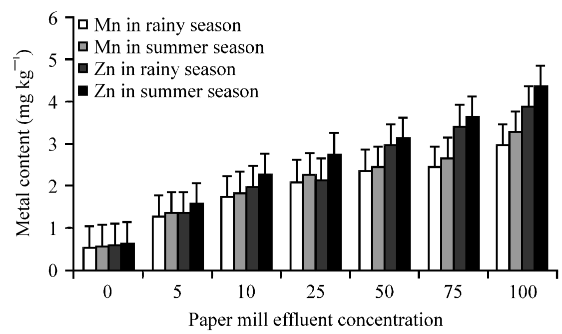

Fig. 8: Content of Mn and Zn in *A. esculentus* after irrigation with paper mill effluent. Error bars are standard error of the mean

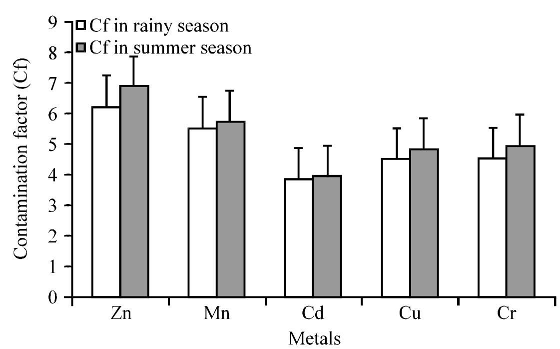

Fig. 9: Contamination factor of various metals in *A. esculentus* after irrigation with paper mill effluent. Error bars are standard error of the mean

could lead to increases of metals in tissues. The Cd, Cr, Cu, Mn and Zn contents in *A. esculentus* was highest with 100% paper mill effluent (Fig. 7, 8). Enrichment of various metals was also observed by Fazeli *et al.* (1998) in paddy crops after paper mill effluent irrigation.

The Contamination factor (Cf) was affected in both seasons (Fig. 9). The Cf of various metals was in order of Zn>Mn>Cu>Cr>Cd in *A. esculentus* after irrigation with paper mill effluent (Fig. 9). The highest contamination factor was for Zn; the least was for Cd in *A. esculentus* with 100% paper mill effluent in both seasons. The micronutrient contents were higher at higher paper mill effluent concentration and likely inhibited growth of *A. esculentus*. The 25% paper mill effluent favored vegetative growth, flowering and maturity of *A. esculentus*. This is likely due to optimal uptake of these micronutrients by crop plants, which supports various biochemical and physiological processes.

CONCLUSION

The present investigation concluded that, paper mill effluent fertigation increased EC, pH, OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, TKN, PO_4^{3-}, SO_4^{2-}, Fe, Zn, Cu, and Mn of the soil in both seasons. Thus, fertigation improved the soil nutrient status and affected the growth of *A. esculentus* in both seasons. The most agronomical growth of *A. esculentus* was observed with 25% concentration of paper mill effluent in both seasons. The growth of *A. esculentus* was inhibited at higher concentrations (50% to 100%) it might be due to presence of more content of heavy metals at these concentrations. Among both seasons, maximum agronomical performance of *A. esculentus* was noted in rainy season. The effluent has potentiality for its use as biofertigant in the form of plant nutrients needed by *A. esculentus* crop plant. Therefore, it can be used as agro-based biofertigant after its appropriate dilution for irrigation purposes for the maximum yield of this crop. Further studies on the agronomic growth and changes in biochemical composition of *A. esculentus* after paper mill effluent irrigation are required.

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