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Distribution, Enrichment and Accumulation of Heavy Metals in Soil and *Trigonella foenum-graecum* L. (Fenugreek) after Fertigation with Paper Mill Effluent

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ABSTRACT

The aim of the study was to investigate distribution, enrichment and accumulation of heavy metals in soil and *Trigonella foenum-graecum* (var. Pusa Early Bunching) after fertigation with paper mill effluent. Doses of paper mill effluent viz. 5%, 10%, 25%, 50%, 75% and 100% were used for fertigation of *T. foenum-graecum* along with bore well water (control). The results 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 *T. foenum-graecum* was increased from 5% to 25% concentration 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 *T. foenum-graecum* 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 enrichment factor (Ef) of various heavy metals was in order of Cd > Mn > Cr > Cu > Zn > Fe for soil and Mn > Cu > Cr > Cd > Zn > Fe for *T. foenum-graecum* plants after fertigation with paper mill effluent. Therefore, paper mill effluent can be used as a biofertigant after appropriate dilution to improve yield of *T. foenum-graecum*.

Keywords: *Trigonella foenum-graecum*; Agronomical Characteristics; Enrichment Factor; Fertigation; Heavy Metals; Paper Mill Effluent

1. Introduction

Industrial or domestic effluent is mostly used for the fertigation of agricultural crops, mainly in urban and peri-urban regions, due to its easy availability, disposal problems and scarcity of fresh water [1,2]. Irrigation with effluents is known to contribute significantly to the heavy metals content of soil as well as crop plants [3-5]. Heavy metals are very harmful because of their non-biodegradable nature, long biological half-lives and their potential to accumulate in different body parts [6-8]. Most of the heavy metals are extremely toxic because of their solubility in water [3,9,10]. Wastewater contains substantial amounts of toxic heavy metals, which create problems [6,11-13]. Excessive accumulation of heavy metals in agricultural soils through wastewater irrigation, may not only result in soil contamination, but also affect food quality and safety [8,14-16].

Heavy metals accumulation in agricultural soils is of increasing worldwide concern and particularly in India with the rapid development of industrialization and urbanization [17-19]. Heavy metals are easily accumulated in the edible parts of leafy vegetables, as compared to grain or fruit crops [20,21]. Vegetables take up heavy metals and accumulate them in their edible and inedible parts in quantities high enough to cause clinical problems both to animals and human beings consuming these metal-rich plants [8,22]. A number of serious health problems can develop as a result of excessive uptake of dietary heavy metals [19,23-25]. Industrial effluent is mostly used for the fertigation of agricultural crops, mainly in

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urban and periurban regions, due to its easy availability, disposal problems and scarcity of fresh water [26,27]. Long term irrigation with effluents is known to contribute significantly to the heavy metals content of soil and increase the chances of their entrance in food chain, and this ultimately causes significant geoaccumulation, bioaccumulation and biomagnifications [13,28].

India has 666 pulp and paper mills, out of which 632 mills are agro-residue based mills [29,30]. They generate a huge amount of wastewater (black liquor) having high biological oxygen demand (BOD) and chemical oxygen demand (COD) values [13,31]. Fenugreek is used as vegetables as well as pulse [32]. The leaves and young pods are used as vegetables and the seeds as condiments. It has also some medicinal value. It prevents constipation removes indigestion stimulates the spleen and is appetizing and diuretic. The leaves are quite rich in protein minerals and Vitamin C [32].

Irrigation of crops with effluents is a very common practice in India due to scarcity of irrigation water [33, 34]. The effect of irrigation with effluents is also studied in many crops to observe the concentration of accumulated metals to which human beings are exposed [5,35, 36]. Heavy metals are easily accumulated in the edible parts of leafy vegetables, as compared to grain or fruit crops [4,7]. Vegetables take up heavy metals and accumulate them in their edible and inedible parts in quantities high enough to cause clinical problems both to animals and human beings consuming these metal-rich plants [16,21]. A number of serious health problems can develop as a result of excessive uptake of dietary heavy metals [12,37].

In recent years, many studies have carried out about effluents quality and its effect on soil and agricultural crops [7,15,38-40]. The researches indicated paper mill industries not only led to accumulation of toxic elements in soil environment, but also increased the risk of accumulation in crop plants [20,35,37]. The present study was conducted with an aim to study the distribution and accumulation of heavy metals in soil and potential of the commonly grown leafy vegetable *Trigonella foenum-graecum* L. (Fenugreek) after fertigation with paper mill effluent.

2. Materials and Methods

2.1. 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). The crop was cultivated in the summer and winter seasons during the year 2010 and 2011. Seven plots (each plot had an area of 9 m^2) were selected for seven treatments of paper mill effluent viz. 0% (control), 10%, 25%, 50%, 75% and 100% for the cultivation of *T. foenum-graecum*. The seven treatments were placed within seven blocks in a randomized complete block design.

2.2. Effluent Collection and Analysis

The effluent samples were collected from the Uttranchal Pulp & Paper Mills (P) Ltd. Haridwar (29˚46'4"N 77˚50'47"E), which produces paper from agricultural waste or residues. Effluent was collected from a settling tank installed on the campus, by the paper mill, to reduce biological oxygen demand (BOD) and solids. The effluxents were collected 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 $\left(CO_3^{2-}\right)$, sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), total Kjeldahl nitrogen (TKN), nitrate $\left(NO_3^{2-}\right)$, phosphate $\left(PO_4^{3-}\right)$, sulphate $\left(SO_4^{2-}\right)$, cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), standard plate count (SPC) and most probable number (MPN) following standard methods [41,42] and used as fertigant.

2.3. Sowing of Seeds and Irrigation Pattern

Seed of *T. foenum-graecum* were sown at the end of February 2010 and 2011 for the summer crop and at the end of October 2010 and 2011 for the winter season crop. Seed of *T. foenum-graecum*, cv. Pusa Early Bunching, were procured from ICAR, Pusa, New Delhi, and sterilized with 0.01% mercuric chloride and soaked in water for 12 hrs. 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.

2.4. Irrigation Pattern, Soil Sampling and Analysis

The plants in each plot were fertigated twice in a month with 50 gallons of paper mill effluent concentrations 5%, 10%, 25%, 50%, 75%, 100% and bore 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 [42].

2.5. Study of Crop Parameters

The agronomic parameters of *T. foenum-graecum* at different stages (0 - 120 days) were determined following standard methods for seed germination, plant height, root length, number of flowers, number of fruits, fruits length and crop yield [43]; dry weight [44]; chlorophyll content [45]; relative toxicity (RT) [46], Leaf Area Index (LAI) [47] and harvest index (HI) [48]. The nutrient quality of *T. foenum-graecum* was determined by using the following parameters; crude protein, crude fiber and the total carbohydrate in dry matter were determined by standard methods [49].

2.6. 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 hr at 145˚C. To this mix 4 ml of $HClO_4$ was added and heated to 240˚C for 1 hr. The mix was cooled and filtered through Whatman # 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 standard methods [41,42]. The enrichment factor (Ef) for metals accumulated in paper mill effluent irrigated soil and *T. foenum-graecum* was calculated following methods [22].

2.7. 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).

3. Results and Discussion

3.1. 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 8.74. 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 [50]. 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 (668.56 mg·L^{-1}), COD (1026.48 mg·L^{-1}), total nitrogen (42.34 mg·L^{-1}), chlorides (426.75 mg·L^{-1}), sulphate (675.82 mg·L^{-1}) and phosphate (51.30 mg·L^{-1}) in paper mill effluent reported by Patterson *et al.* [51]. In the case of metals, the contents of Cd, Cr Cu, Fe, Mn and Zn were higher than permissible limits for industrial effluent [50]. The content of these metals in paper mill effluent were also higher then the content of Cd (9.36 mg·L^{-1}), Cr (16.46 mg·L^{-1}) Cu (10.52 mg·L^{-1}) and Zn (10.64 mg·L^{-1}), in paper mill effluent reported by Singh *et al.* [52].

3.2. 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 (120 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 (**Tables 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 [29,51]. WHC and BD were reduced from their initial (control) values 43.12% and 1.41 gm·cm^{-3} to 42.34% and 1.40 gm·cm^{-3} respectively with 100% paper mill effluent concentration. The pH of the soil was turned alkaline to more alkaline (8.89 and 8.98) 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 [29,31]. 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 *T. foenum-graecum* 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 *T. foenum-graecum* cultivated soil in both seasons (**Tables 5** and **6**). Irrigation with 100% paper mill effluent

Table 1. Physico-chemical and microbiological characteristics of paper mill effluent (PME).

Parameter	Effluent concentration (%)							BIS[b] for irrigation water
	0 (BWW)[a]	5	10	25	50	75	100	
TDS ($mg \cdot L^{-1}$)	245.67	1468.50	1896.80	2412.37	2688.65	2998.77	4086.00	1900
EC ($dS \cdot m^{-1}$)	0.32	2.14	2.92	3.98	4.18	4.96	6.63	-
pH	7.58	7.68	7.79	7.86	7.97	8.24	8.74	5.5-9.0
DO ($mg \cdot L^{-1}$)	8.48	5.34	4.76	3.98	2.45	1.74	Nil	-
BOD ($mg \cdot L^{-1}$)	3.76	78.90	168.56	342.43	632.48	964.57	1256.84	100
COD ($mg \cdot L^{-1}$)	5.84	172.34	324.66	842.78	1556.76	2256.92	2976.40	250
Cl^- ($mg \cdot L^{-1}$)	62.45	83.66	142.70	264.60	512.45	785.54	970.50	500
HCO_3^- ($mg \cdot L^{-1}$)	264.70	293.64	312.44	424.88	879.90	996.45	1034.56	-
CO_3^{2-} ($mg \cdot L^{-1}$)	110.88	128.45	262.70	368.97	670.44	825.60	934.65	-
Na^+ ($mg \cdot L^{-1}$)	8.34	32.44	65.86	142.34	294.30	388.55	507.32	-
K^+ ($mg \cdot L^{-1}$)	4.76	18.61	39.44	84.50	172.80	234.72	287.34	-
Ca^{2+} ($mg \cdot L^{-1}$)	29.60	68.90	127.78	264.47	448.90	593.90	798.30	200
Mg^{2+} ($mg \cdot L^{-1}$)	12.78	24.56	50.76	112.30	182.56	258.45	326.72	-
TKN ($mg \cdot L^{-1}$)	19.10	38.60	78.20	104.76	209.33	312.44	432.65	100
NO_3^{2-} ($mg \cdot L^{-1}$)	34.56	58.40	119.34	198.34	267.88	487.20	562.34	100
PO_4^{3-} ($mg \cdot L^{-1}$)	0.06	10.56	22.12	56.70	115.50	185.42	234.50	-
SO_4^{2-} ($mg \cdot L^{-1}$)	78.90	134.80	202.50	422.41	845.68	1460.20	1696.40	1000
Fe^{2+} ($mg \cdot L^{-1}$)	0.42	1.19	2.42	4.98	10.04	15.78	20.12	1.0
Cd ($mg \cdot L^{-1}$)	BDL[c]	0.72	1.43	2.98	5.44	7.24	10.90	15
Cr ($mg \cdot L^{-1}$)	BDL	1.32	2.68	5.67	10.34	16.78	21.34	2.00
Cu ($mg \cdot L^{-1}$)	BDL	1.19	2.39	5.93	11.23	18.43	22.49	3.00
Mn ($mg \cdot L^{-1}$)	0.02	0.72	1.45	4.26	7.70	10.61	15.45	1.00
Zn ($mg \cdot L^{-1}$)	0.04	0.60	1.22	3.12	6.26	8.42	12.56	2.00
SPC (SPC mL^{-1})	4.8×10^1	6.8×10^5	5.3×10^6	7.3×10^8	8.6×10^9	9.2×10^{10}	9.7×10^{13}	10000
MPN (MPN 100 mL^{-1})	3.6×10^1	4.7×10^4	6.4×10^5	8.1×10^6	5.7×10^8	6.8×10^9	6.2×10^{11}	5000

[a]BWW = bore well water; [b]BIS = bureau of Indian standard; [c]BDL = below detection limit; 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 $P \le 0.05$ or $P \le 0.01$, 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 $P \le 0.05$ or $P \le 0.01$, 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	**	**	**	**	**

^{ns}, *, **Non-significant or significant at $P \le 0.05$ or $P \le 0.01$, ANOVA.

had the most reduction in WHC, BD; and increase in EC, OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, TKN, PO_4^{3-}, SO_4^{2-}, Cd, Cr, Cu, Mn and Zn in both seasons (**Tables 5** and **6**). The findings were very much in accordance with Patterson *et al.* [51].

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

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

Season × %PME		EC ($dS \cdot m^{-1}$)	pH	OC ($mg \cdot L^{-1}$)	Na^+ ($mg \cdot L^{-1}$)	K^+ ($mg \cdot L^{-1}$)	Ca^{2+} ($mg \cdot L^{-1}$)	Mg^{2+} ($mg \cdot L^{-1}$)
Winter	0	2.17	7.67	0.42	19.34	165.40	18.54	1.82
	5	2.64^{ns}	8.17^{ns}	1.69^{*}	26.44^{ns}	175.39^{ns}	39.20^{ns}	4.34^{ns}
	10	2.76^{ns}	8.32^{ns}	3.77^{*}	28.93^{*}	184.69^{ns}	47.80^{ns}	7.89^{ns}
	25	2.96^{*}	8.41^{ns}	5.76^{**}	33.42^{*}	196.86^{*}	87.45^{*}	11.30^{*}
	50	3.02^{*}	8.57^{*}	6.87^{**}	39.67^{*}	218.73^{*}	129.50^{*}	14.42^{*}
	75	3.15^{*}	8.82^{*}	8.45^{**}	44.54^{**}	234.40^{**}	152.67^{*}	18.55^{*}
	100	3.27^{**}	8.89^{*}	10.12^{**}	50.72^{**}	239.86^{**}	175.68^{**}	25.90^{*}
Summer	0	2.18	7.69	0.44	19.88	165.70	18.89	1.83
	5	2.78^{ns}	8.22^{ns}	1.74^{*}	29.60^{ns}	182.20^{ns}	42.57^{ns}	4.54^{ns}
	10	2.94^{ns}	8.36^{ns}	4.86^{*}	31.87^{*}	206.77^{ns}	50.56^{ns}	9.05^{ns}
	25	3.07^{*}	8.47^{ns}	6.75^{**}	36.75^{*}	214.79^{*}	92.55^{*}	13.24^{*}
	50	316^{*}	8.69^{*}	8.98^{**}	42.32^{*}	227.56^{*}	135.65^{*}	16.34^{*}
	75	3.26^{*}	8.87^{*}	9.78^{**}	47.50^{**}	239.54^{**}	161.34^{*}	20.11^{*}
	100	3.32^{**}	8.98^{*}	11.56^{**}	54.66^{**}	248.70^{**}	180.40^{**}	28.76^{*}

^{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 *T. foenum-graecum* in both seasons.

Season × %PME		TKN ($mg \cdot L^{-1}$)	PO_4^{3-} ($mg \cdot L^{-1}$)	SO_4^{2-} ($mg \cdot L^{-1}$)	Fe^{2+} ($mg \cdot L^{-1}$)	Cd ($mg \cdot L^{-1}$)	Cr ($mg \cdot L^{-1}$)	Cu ($mg \cdot L^{-1}$)	Mn ($mg \cdot L^{-1}$)	Zn ($mg \cdot L^{-1}$)
Winter	0	42.23	55.70	78.90	2.86	0.76	0.87	2.12	0.46	1.11
	5	62.96^{ns}	72.76^{ns}	80.77^{ns}	4.09^{*}	2.30^{*}	1.92^{*}	3.88^{*}	1.29^{*}	1.90^{*}
	10	74.50^{**}	82.55^{ns}	92.30^{ns}	5.04^{*}	2.89^{*}	3.02^{*}	4.89^{*}	1.43^{*}	2.54^{*}
	25	145.76^{**}	102.20^{*}	103.54^{*}	7.10^{**}	3.94^{**}	5.12^{**}	6.10^{**}	2.07^{**}	2.78^{**}
	50	217.80^{**}	118.60^{**}	127.77^{*}	8.78^{**}	5.14^{**}	6.09^{**}	7.21^{**}	2.96^{**}	3.98^{**}
	75	278.56^{**}	128.77^{**}	138.90^{**}	9.23^{**}	6.21^{**}	6.88^{**}	8.44^{**}	3.62^{**}	4.32^{**}
	100	304.66^{**}	138.79^{**}	147.84^{**}	11.20^{**}	7.34^{**}	7.93^{**}	9.87^{**}	4.35^{**}	5.11^{**}
Summer	0	42.88	56.12	78.98	2.86	0.78	0.88	2.13	0.48	1.13
	5	68.87^{ns}	78.92^{ns}	86.60^{ns}	4.20^{*}	2.67^{*}	2.11^{*}	3.98^{*}	1.42^{*}	1.98^{*}
	10	82.45^{**}	88.96^{*}	97.56^{*}	5.60^{*}	3.43^{*}	3.25^{*}	5.11^{*}	1.96^{*}	2.78^{*}
	25	153.60^{**}	108.84^{*}	110.24^{*}	7.44^{**}	4.56^{**}	5.60^{**}	6.34^{**}	2.12^{**}	3.64^{**}
	50	224.78^{**}	124.69^{**}	134.80^{*}	9.32^{**}	5.78^{**}	6.87^{**}	7.50^{**}	3.05^{**}	4.12^{**}
	75	286.80^{**}	134.56^{**}	146.45^{**}	10.94^{**}	6.54^{**}	7.45^{**}	8.56^{**}	3.77^{**}	4.78^{**}
	100	312.87^{**}	145.60^{**}	156.70^{**}	12.67^{**}	7.80^{**}	8.32^{**}	10.33^{**}	4.56^{**}	5.67^{**}

^{ns}, *, **Non-significant or significant at $P < 0.01$; Least Squares Means analysis.

effluent. Kumar [29] 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 enrichment factor (Ef) of the metals indicated that Cd was highest while Fe was lowest in both seasons after irrigation with 100% paper mill effluent. The Ef of metals were in the order of Cd > Mn

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	Winter	−0.97
	Summer	−0.98
Paper mill effluent versus soil BD	Winter	−0.95
	Summer	−0.96
Paper mill effluent versus soil EC	Winter	+0.87
	Summer	+0.88
Paper mill effluent versus soil pH	Winter	+0.90
	Summer	+0.91
Paper mill effluent versus soil OC	Winter	+0.97
	Summer	+0.98
Paper mill effluent versus soil Na^+	Winter	+0.95
	Summer	+0.96
Paper mill effluent versus soil K^+	Winter	+0.96
	Summer	+0.97
Paper mill effluent versus soil Ca^{2+}	Winter	+0.91
	Summer	+0.92
Paper mill effluent versus soil Mg^{2+}	Winter	+0.96
	Summer	+0.97
Paper mill effluent versus soil TKN	Winter	+0.98
	Summer	+0.99
Paper mill effluent versus soil PO_4^{3-}	Winter	+0.95
	Summer	+0.94
Paper mill effluent versus soil SO_4^{2-}	Winter	+0.99
	Summer	+0.99
Paper mill effluent versus soil Fe^{2+}	Winter	+0.97
	Summer	+0.98
Paper mill effluent versus soil Cd	Winter	+0.97
	Summer	+0.96
Paper mill effluent versus soil Cr	Winter	+0.98
	Summer	+0.97
Paper mill effluent versus soil Cu	Winter	+0.98
	Summer	+0.99
Paper mill effluent versus soil Mn	Winter	+0.94
	Summer	+0.95
Paper mill effluent versus soil Zn	Winter	+0.96
	Summer	+0.97

> Cr > Cu > Zn > Fe after irrigation with paper mill effluent in both seasons (**Figure 1**). The concentrations of metals were higher in soil irrigated with effluent than in

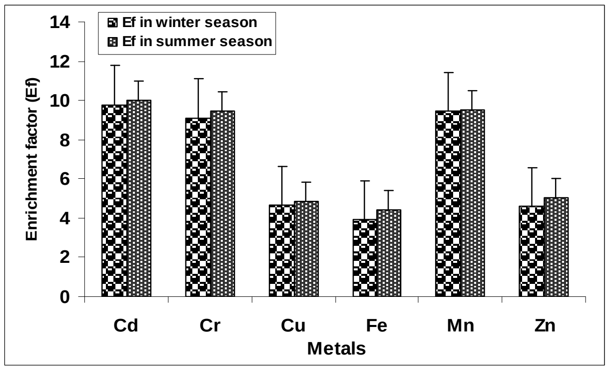

Figure 1. Enrichment factor of the metals in soil after fertigation with paper mill effluent. Error bars are standard error of the mean.

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.* [4] in soil after paper mill effluent irrigation.

3.3. Effect of Paper Mill Effluent on Seed Germination of *T. foenum-graecum*

At 0 - 15 days after sowing, the maximum seed germination (98% and 96%) was for with control and the least (87% and 86%) was due to treatment with 100% paper mill effluent (**Figure 2**). Germination was negatively correlated ($r = -0.96$ and $r = -0.97$) 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 concentration and their interaction with season affected seed germination of *T. foenum-graecum*, but not relative toxicity (**Table 8**).

The maximum relative toxicity (110.34% and 113.95%) of paper mill effluent against germination was for the 100% paper mill effluent (**Figure 3**) and it was positively correlated ($r = +0.50$ and $r = +0.52$) with paper mill effluent concentrations in both seasons. The findings are very much in accordance with Medhi *et al.* [53] reported that the germination of green gram (*Brassica campestris* L. and *Pisum sativum* L.) was decreased as concentration of the paper mill effluent increased from 0 to 100%. The findings were also supported by Reddy and Borse [32].

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 *T. foenum-graecum* 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.

Table 8. ANOVA for effect of paper mill effluent on germination and vegetative growth of *T. foenum-graecum*.

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 $P \leq 0.05$, ANOVA.

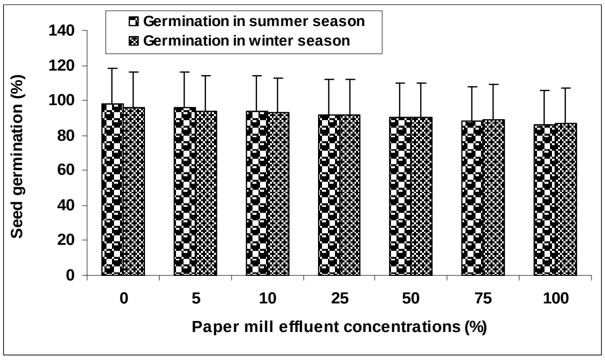

Figure 2. Seed germination of *T. foenum-graecum* after fertigation with paper mill effluent. Error bars are standard error of the mean.

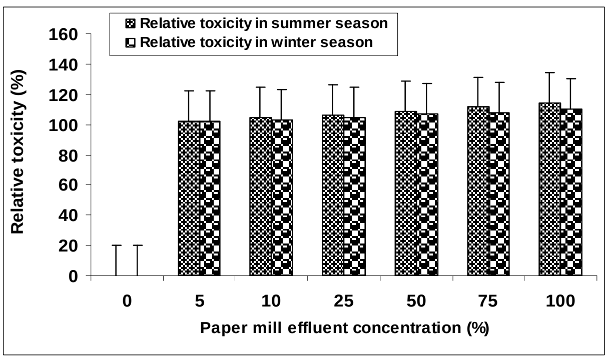

Figure 3. Relative toxicity of paper mill effluent against seed germination of *T. foenum-graecum*. Error bars are standard error of the mean.

The mechanism of inhibition of seed germination by NaCl may be related to radical 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 [3,51]. 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 [30,52].

3.4. Effect of Paper Mill Effluent on Vegetative Growth of *T. foenum-graecum*

Vegetative growth at 45 days after sowing was affected in both seasons (**Table 8**). Average plant height (24.25 and 29.45 cm), root length (7.89 and 8.67 cm), dry weight (1.42 and 1.47 g), chlorophyll content (3.48 and 3.52 mg./g.f.wt) and LAI/plant (3.31 and 3.34) of *T. foenum-graecum* were observed with control while plant height (30.86 and 32.75 cm), root length (10.36 and 11.48 cm), dry weight (1.67 and 1.72 g), chlorophyll content (3.61 and 3.65 mg./g.f.wt) and LAI/plant (3.36 and 3.39) of *T. foenum-graecum* were noted with 100% paper mill effluent in both seasons.

Maximum plant height (36.75 and 39.89 cm), root length (11.78 and 12.14 cm), dry weight (1.87 and 1.92 g), chlorophyll content (3.86 and 3.92 mg./g.f.wt) and LAI/plant (3.56 and 3.64) of *T. foenum-graecum* were due to treatment with the 25% concentration of paper mill effluent in both seasons. The findings were also supported by Reddy and Borse [32]. The ANOVA indicated that paper mill effluent concentration significantly ($P < 0.05$) affected plant height, and chlorophyll content of *T. foenum-graecum* (**Table 8**). Season had no effect on plant height, root length, dry weight and LAI of *T. foenum-graecum*. The interaction of season and paper mill effluent concentrations only affected plant height and chlorophyll content of T. foenum-graecum (**Table 8**).

Plant height, root length, dry weight, chlorophyll content and LAI/plant of *T. foenum-graecum* were positively correlated with paper mill effluent concentrations in both seasons (**Table 9**). Reddy and Borse [32] reported the maximum chlorophyll content in *T. foenum-graecum* at 25% concentration of distillery effluent. Medhi *et al.* [53] reported that paper mill effluent irrigation increase chlorophyll and protein contents in Indian mustard plants (*Brassica campestris* 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 Reddy and Borse [32] who reported that the growth of *T. foenum-graecum* (L.) decreased when concentration of paper mill increased.

Vegetative growth of *T. foenum-graecum* 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 *T. foenum-graecum*. Vegetative growth is associated with development of new shoots, twigs, leaves and leaf area. Plant height, root length, dry weight and LAI/plant of *T. foenum-graecum* 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 [29,32]. 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 with chlorophyll synthesis [45]. The 25% paper mill effluent concentration contains optimum contents of nutrients required for maximum vegetative growth of *T. foenum-graecum*.

3.5. Effect of Paper Mill Effluent on Flowering of *T. foenum-graecum*

Numbers of flowers decreased as paper mill effluent concentration decreased (**Table 9**). At flowering stage (60 days after sowing) the maximum flowers (33.00 and 36.00) was noted with 25% paper mill effluent in both seasons. Numbers of flowers/plant 23.00 and 25.00 were with control and 27.00 to 29.00 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 [29,31,54]. Flowering of *T. foenum-graecum* 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 [29,31,32,54].

3.6. Effect of Paper Mill Effluent on Maturity of *T. foenum-graecum*

At maturity stage (120 days after showing) the most fruits/plants (31.00 and 34.00), fruit length (9.36 cm and 9.76 cm) yield/plant (19.14 and 22.39 g), and HI (1023.52 and 1166.14%) of *T. foenum-graecum* were

Table 9. Coefficient of correlation (*r*) between paper mill effluent and *T. foenum-graecum* in both seasons.

Paper mill effluent/*T. foenum-graecum*	Season	*r*-value
Paper mill effluent versus shoot length	Winter	+0.67
	Summer	+0.66
Paper mill effluent versus root length	Winter	+0.18
	Summer	+0.17
Paper mill effluent versus dry weight	Winter	+0.27
	Summer	+0.28
Paper mill effluent versus chlorophyll content	Winter	+0.29
	Summer	+0.30
Paper mill effluent versus LAI	Winter	+0.47
	Summer	+0.49
Paper mill effluent versus no. of flowers/plant	Winter	+0.48
	Summer	+0.47
Paper mill effluent versus no. of fruits	Winter	+0.58
	Summer	+0.57
Paper mill effluent versus fruit length	Winter	+0.48
	Summer	+0.49
Paper mill effluent versus crop yield/plant	Winter	+0.14
	Summer	+0.13
Paper mill effluent versus harvest index	Winter	+0.47
	Summer	+0.43
Paper mill effluent versus Cd	Winter	+0.98
	Summer	+0.99
Paper mill effluent versus Cr	Winter	+0.97
	Summer	+0.96
Paper mill effluent versus Cu	Winter	+0.98
	Summer	+0.99
Paper mill effluent versus Mn	Winter	+0.99
	Summer	+0.98
Paper mill effluent versus Zn	Winter	+0.96
	Summer	+0.97

Table 10. ANOVA for effect of paper mill effluent on flowering and maturity stage of *T. foenum-graecum*.

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.

with the 25% paper mill effluent in both seasons. Numbers of fruits/plant, crop yield/plant and harvest index (HI) of *T. foenum-graecum* 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 *T. foenum-graecum* were not affected by season, paper mill effluent concentration and their interaction (**Table 10**). The number of fruits/plants (21.00 and 23.00), fruit length (6.10 cm and 6.32 cm) yield/plant (12.45 and 13.52 g), and HI (876.76 and 919.72%) of *T. foenum-graecum* were with the control while with 100% paper mill effluent the fruits/plants (25.00 and 27.00), fruit length (8.24 cm and 8.29 cm) yield/plant (15.42 and 16.34 g), and HI (923.35% and 950.00%) of *T. foenum-graecum* 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 [29,52]. The K, Fe, Mg and Mn contents could benefit pod formation and yield of as it does for fenugreek (*T. foenum-graecum L.*) as reported by Reddy and Borse [32]. The 25% paper mill effluent favored fruits formation and crop yield of *T. foenum-graecum*. 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 *T. foenum-graecum.*

3.7. Effect on Biochemical Constituents and Metals in *T. foenum-graecum*

The content of various metals 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 T. foenum-graecum (**Table 11**). Maximum crude proteins, crude fiber and crude carbohydrates were recorded with 25% paper mill effluent concentrations in both seasons (**Figures 4-6**). Content of crude proteins (r = +0.45 and r = +0.47), crude fiber (r = +0.37 and r = +43) and crude carbohydrates (r = +0.59 and r = +0.61) 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, Fe, Mn and Zn contents in *T. foenum-graecum*. Increased irrigation frequency could lead to increases of metals in tissues. The Cd, Cr, Cu, Fe, Mn and Zn contents in *T. foenum-graecum* was highest with 100% paper mill effluent (**Figures 7**, **8**). Enrichment of various metals was also observed by Fazeli *et al.* [4] in paddy crops after paper mill effluent irrigation. The findings are very much in accordance with Pathak *et al.* [9,10].

The enrichment factor (Ef) was affected in both sea-

Table 11. ANOVA for effect of paper mill effluent on flowering and maturity stage of *T. foenum-graecum*.

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 $P \le 0.05$ or $P \le 0.01$, ANOVA.

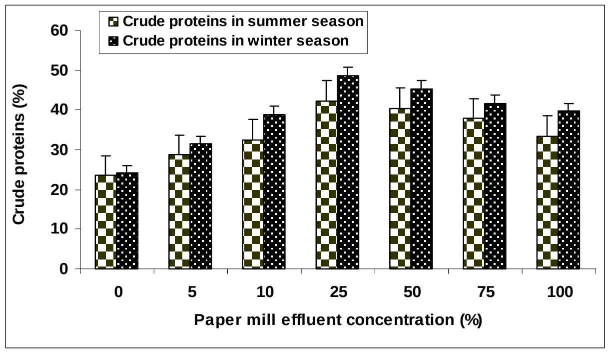

Figure 4. Crude proteins in *T. foenum-graecum* after fertigation with paper mill effluent. Error bars are standard error of the mean.

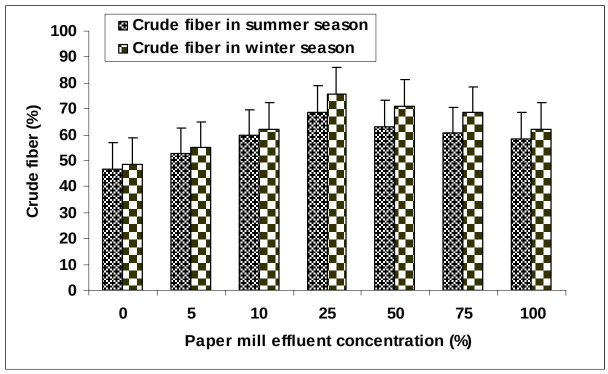

Figure 5. Crude fiber in *T. foenum-graecum* after fertigation with paper mill effluent. Error bars are standard error of the mean.

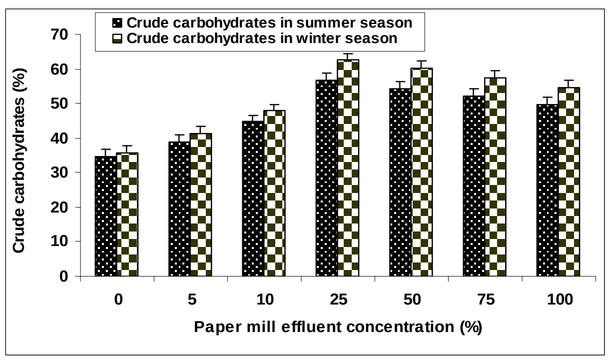

Figure 6. Crude carbohydrates in *T. foenum-graecum* after fertigation with paper mill effluent. Error bars are standard error of the mean.

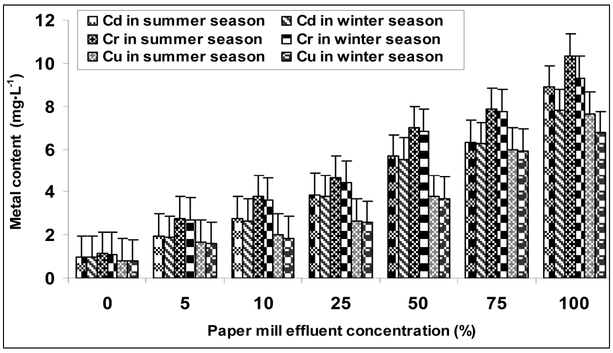

Figure 7. Content of Cd, Cr and Cu in *T. foenum-graecum* after fertigation with paper mill effluent. Error bars are standard error of the mean.

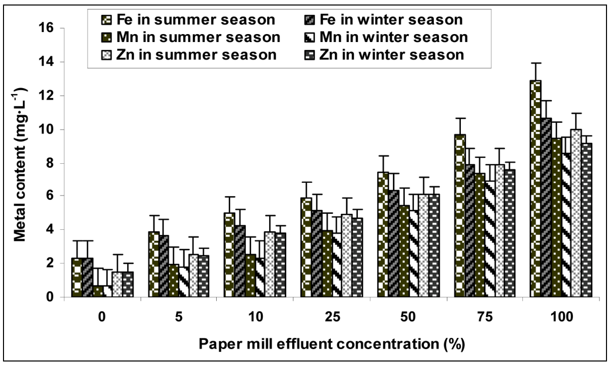

Figure 8. Content of Fe, Mn and Zn in *T. foenum-graecum* after fertigation with paper mill effluent. Error bars are standard error of the mean.

sons (**Figure 9**). The Ef of various metals was in order of Mn > Cu > Cr > Cd > Zn > Fe in *T. foenum-graecum* after irrigation with paper mill effluent (**Figure 9**). The highest enrichment factor was for Mn; the least was for Fe in *T. foenum-graecum* with 100% paper mill effluent in both seasons. The metals contents were higher at higher paper mill effluent concentration, and likely inhibited growth of *T. foenum-graecum*. The 25% paper mill effluent favored vegetative growth, flowering and maturity of *T. foenum-graecum*. This is likely due to optimal uptake of these micronutrients by crop plants, which supports various biochemical and physiological processes.

4. 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 *T. foenum-graecum* in both seasons. The most agronomical growth of *T. foenum-graecum* was observed with 25% concentration of paper mill effluent in both seasons. The growth of *T. foenum-graecum* was inhibited at higher concentrations (50% to 100%), it might be due to the presence of more content of heavy metals at these concentrations. The enrichment factor (Ef) of various heavy metals was in order of Cd > Mn > Cr > Cu > Zn > Fe for soil and Mn > Cu > Cr > Cd > Zn > Fe for *T. foenum-graecum* plants after fertigation with paper mill effluent. Among both seasons, maximum agronomical performance of *T. foenum-graecum* was noted in winter season. The effluent has potentiality for its use as biofertigant in the form of plant nutrients needed by *T. foenum-graecum* 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 *T. foenum-graecum* after paper mill effluent irrigation are required.

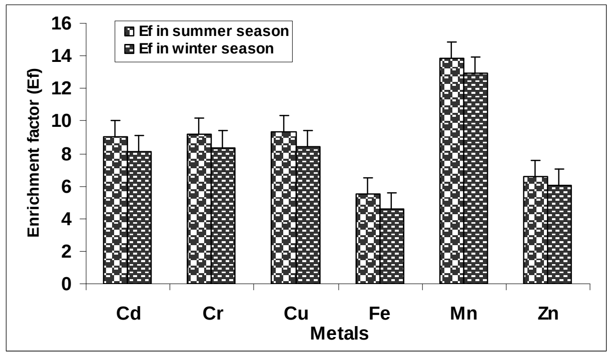

Figure 9. Enrichment factor of various metals in *T. foenum-graecum* after fertigation with paper mill effluent. Error bars are standard error of the mean.

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