

Pearl millet (*Pennisetum Glaucum* L.) response after ferti-irrigation with sugar mill effluent in two seasons

Vinod Kumar · A. K. Chopra

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Abstract

Background The disposal of sugar mill effluent has become a major problem in India due to generation of huge volume of effluent. The value of wastewater for crop production has been recognized in many countries, including India. The effluents not only contain nutrients that stimulate growth of many crops, but also may have various toxic chemicals, metals, metallic oxides along with nitrogenous and phosphate compounds, which may affect various agronomical characteristics of crop plants. The present investigation was conducted to assess the agro-potentiality of agro-based sugar mill effluent as ferti-irrigant, and an alternative of irrigation water. Six plots were selected for six treatments of sugar mill effluent viz. 0 % (control), 20, 40, 60, 80, and 100 % for the fertigation of *Pennisetum glaucum* L., cv. Nandi 35. *P. glaucum* was grown, fertigated with effluent till harvest and effect of effluent fertigation on the soil and agronomical characteristics of *P. glaucum* were analyzed.

Results The fertigant concentration produced changes in electrical conductivity (EC), pH, organic carbon (OC), sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), total Kjeldahl nitrogen (TKN), phosphate (PO_4^{3-}), sulfate (SO_4^{2-}), iron (Fe), cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), and zinc (Zn) of the soil in both seasons. The agronomic performance of *P. glaucum* increased from 20 to 40 % in both seasons compared to controls. The accumulation of heavy metals increased in soil and *P. glaucum* from 20 to 100 % sugar mill effluent concentrations in both seasons. Biochemical components like

crude proteins, crude fiber, and crude carbohydrates were found maximum with 40 % sugar mill effluent in both seasons. The contamination factor (Cf) of various metals were in the order of $\text{Mn} > \text{Zn} > \text{Cu} > \text{Cd} > \text{Cr}$ for soil and $\text{Mn} > \text{Zn} > \text{Cu} > \text{Cr} > \text{Cd}$ for *P. glaucum* in both seasons after fertigation with sugar mill effluent. Sugar mill effluent irrigation increased nutrients in the soil and affected the growth of *P. glaucum* in both seasons.

Conclusions It appears that sugar mill effluent can be used as a biofertilizer after appropriate dilution to improve yield of *P. glaucum*.

Keywords *Pennisetum glaucum* · Sugar mill effluent · Fertigation · Heavy metals · Rainy and summer season

Nomenclature

°C	Degree centigrade
cm	Centimeter
dS cm^{-1}	Desi Siemens per centimeter
g	Gram
gm cm^{-3}	Gram per cubic centimeter
kg ha^{-1}	Kilogram per hectare
ml	Milliliter
mg L^{-1}	Milligram per liter
mg Kg^{-1}	Milligram per kilogram
mg (g f wt)^{-1}	Milligram per gram fresh weight
m^2	Square meter
%	Percentage
NTU	Nephelometric turbidity unit

Introduction

India is one of the largest producers of sugar in the world and presently has nearly 650 sugar mills that produce about

V. Kumar (✉) · A. K. Chopra
Agro-ecology and Pollution Research Laboratory, Department of
Zoology and Environmental Science, Gurukula Kangri
University, Haridwar, Uttarakhand 249404, India
e-mail: drvksorwal@gmail.com

15 million tons of sugar and 13 million tons of molasses (spent wash) per year (Roy et al. 2007; Ezhilvannan et al. 2011). The sugar mill effluent is mainly discharged from floor, wastewater, and condensate water formed by leakage (Rathore et al. 2000; Ezhilvannan et al. 2011). The disposal of polluted wastewater is one of the main problems of today to be faced in the future with its increased adverse effects (Bharagava et al. 2008; Moazzam et al. 2012). Most of the sugar mills are discharging their effluent into the environment without any treatment (Borole and Patil 2004). It has also been reported that sugar mill effluent contains a high magnitude of pollution load and caused adverse effects on soil and biological system (Arindam and Prasad 1999; Ayyasamy et al. 2008). The effluent constitutes a number of physico-chemical elements of suspended and dissolved solids with the high amount of biological oxygen demand (BOD), chemical oxygen demand (COD), chlorides, sulfate, nitrates, calcium, magnesium, and metals (Rathore et al. 2000; Roy et al. 2007).

In addition to that, some traceable amount of heavy metals such as zinc, copper, and lead is usually present in the sugar mill effluent. The presence of these chemicals in large quantities in the effluent not only affects plant growth but also collapses the soil properties when used for irrigation (Al-Jaloud et al. 1995; Roy et al. 2007; Ayyasamy et al. 2008). Therefore, the effluent can be applied for productive uses since it contains nutrients that have the potential for use in agriculture (Kumar and Chopra 2012; Chopra et al. 2012). In agriculture, irrigation water can affect soil characteristics and agricultural crop growth (Almodares and Sharif 2007; Kumar and Chopra 2010). Besides, the use of effluent reduces fertilizer and irrigation water cost as it is available without any cost and is rich in various plant nutrients (Kumar and Chopra 2012).

Irrigation with effluents increases accumulation of metals in soil, and increases chances of their entrance in food chain (Chopra et al. 2009). Thus, contamination of agricultural soils with metals can pose long-term environmental problems, and is not without health implications (Ferguson 1990; Chopra et al. 2009). The amounts of metals mobilized in the soil environment is a function of pH, clay content, organic matter, cation exchange capacity, and other soil properties making each soil unique in terms of pollution management (Baruah et al. 1993; Kumar and Chopra 2012). The metals Cu, Fe, Ni, Zn, and other trace elements are important for proper functioning of biological systems, and their deficiency, or excess, could lead to a number of disorders (Chopra et al. 2009). Metals are capable of forming insoluble complex compounds with soil organic matter, and contents of Cd, Cu, Ni, Mn, and Zn are dependent on the pH of soil solution and soil organic matter (Kim and Kim 1999). Soil type is one of the most important factors to determine the metals' content of food

plants (Itanna 2002; Roy et al. 2007). Metals' content in plants can also be affected by application of fertilizers or irrigation with effluent (Ferguson 1990; Ayyasamy et al. 2008).

Pearl millet is grown on more than 29 million ha in the arid and semi-arid tropical regions of Asia, Africa, and Latin America. Pearl millet has about the same nutritive quality as corn for domestic animals (Oyen and Andrews 1996; Moazzam et al. 2012). Thus, it is widely used for food and fodder all over the world and is considered as fifth most important cereal crop after wheat, maize, rice, and barley. Pearl millet biomass is variously used for the production of energy, fiber, or paper, as well as for syrup and animal feed in several regions (Radhouane 2008; Yadav and Bidinger 2008). In India, pearl millet is the fourth most important food crop, and is mostly cultivated in the northwestern states of the country. India is the largest producer of pearl millet in Asia, both in terms of area (about 9 million ha) and production (8.3 million tons) with an average productivity of 930 kg/ha during the past three years. From the early 1980 s, the pearl millet area in India declined by 22 %, due to inappropriate irrigation facilities (Moazzam et al. 2012). It has the ability to tolerate and survive under adverse conditions of intermittent and continuing drought (Yadav and Bidinger 2008). Therefore, pearl millet has received considerable attention during the last years as an alternative source for food, fodder, and energy production (Oyen and Andrews 1996).

In some reports, characteristics of the effluent of industries and agronomic properties of various crop plants have been determined (Hill et al. 1999; Nadia El-Sawaf 2005; Mendoza et al. 2006; Sakellariou et al. 2007; Moazzam et al. 2012). 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. 2004). Use of industrial effluents on cultivation of *P. glaucum* is receiving attention (Moazzam et al. 2012) but additional information is required on how this crop responds to various concentrations of different types of effluents. The investigation was undertaken to study responses of *P. glaucum* ferti-irrigated with sugar mill effluent.

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 determine the effects of ferti-irrigation with sugar mill

effluent on *P. glaucum*. Six plots (each plot had an area of 9 m²) were selected for six treatments of sugar mill effluent viz. 0 % (control), 20, 40, 60, 80, and 100 % for the cultivation of *P. glaucum*. The six treatments were placed within each of the six blocks in a randomized complete block design.

Sowing of seeds of *P. glaucum*

Seeds of *P. glaucum* were sown at the end of April 2010 and 2011 for the summer season crop and at the end of July 2010 and 2011 for the rainy season crop. Seeds of *P. glaucum*, cv. Nandi 35, were procured from Indian Council of Agriculture Research (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 the 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.

Effluent collection and analysis

The effluent samples were collected from the R.B.N.S. Sugar mill, Laksar, Haridwar (Uttarakhand), which produces sugar from sugar cane at the rate of 150 ton sugar per day. Effluent collected from a settling tank was installed in the campus by the sugar mill to reduce BOD and solids from the effluent in plastic containers. It was brought to the laboratory and analyzed for total dissolved solids (TDS), pH, EC, dissolved oxygen (DO), BOD, COD, chlorides (Cl⁻), bicarbonates (HCO₃⁻), carbonates (CO₃²⁻), Na⁺, K⁺, Ca²⁺, Mg²⁺, TKN, nitrate (NO₃²⁻), PO₄³⁻, SO₄²⁻, Fe, Cd, Cr, Cu, Mn, Zn, standard plate count (SPC), and most probable number (MPN) following standard methods (APHA 2005; Chaturvedi and Sankar 2006) and used as fertigan.

Irrigation pattern, soil sampling, and analysis

The soil in each plot was fertigated twice in a month with 50 gallons of sugar mill effluent with 20, 40, 60, 80, and 100 % along with bore well water as the control. The soil was analyzed prior to planting and after harvest for various physico-chemical parameters: bulk density (BD), water holding capacity (WHC), soil texture, soil pH, EC, OC, Na⁺, K⁺, Ca²⁺, Mg²⁺, TKN, PO₄³⁻, SO₄²⁻, Fe²⁺, Cd, Cr, Cu, Mn, and Zn determined following standard methods (Chaturvedi and Sankar 2006).

Study of crop parameters

The agronomic parameters at different stages (0–90 days) were determined following standard methods for seed

germination, plant height, root length, number of leaves per plant, number of tillers, spikes length, and crop yield (Radhouane 2008); fresh and dry weight (Milner and Hughes 1968); chlorophyll content (Porra 2002); relative toxicity (RT) (Chapagain 1991); leaf area index (LAI) (Denison and Russotti 1997); and harvest index (HI) (Sinclair 1998). The nutrient quality of the crop was determined by using the following parameters: crude protein (4.204 Anonymous 1980), crude fiber (4.601 Anonymous 1980), and the total carbohydrate in dry matter was determined by the anthrone reagent method (Cerning and Guilhot 1973).

Extraction of metals and their analysis

For heavy metal analysis, a 5–10 ml sample of sugar mill effluent, and 0.5–1.0 g of air dried soil or plants were digested in tubes with 3 ml of conc. HNO₃ digested in an electrically heated block for 1 h at 145 °C. To this mix, 4 ml of HClO₄ was added and heated to 240 °C for 1 h. The mixture was cooled and filtered through Whatman # 42 filter paper and made with 50 ml and used for analysis. Heavy metals were analyzed using an Atomic absorption spectrophotometer (PerkinElmer, Analyst 800 AAS, Gen Tech Scientific Inc., Arcade, NY) following methods of APHA (2005) and Chaturvedi and Sankar (2006). The contamination factor (Cf) for heavy metals accumulated in sugar mill effluent irrigated soil and *P. glaucum* was calculated following Håkanson (1980).

Data analysis

Data were analyzed with SPSS (ver. 12.0, SPSS Inc., Chicago, Ill.). Data were subjected to two-way ANOVA. Duncan's multiple range test was also performed to determine that the difference was significant or non significant. 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 effluent

The values of physico-chemical and microbiological parameters varied over sugar mill effluent concentrations (Table 1). The sugar mill effluent was highly alkaline with a pH 8.98. The alkaline nature of the sugar mill effluent might be due to the presence of higher concentrations of

Table 1 Physico-chemical and microbiological characteristics of R.B.N.S. sugar mill effluent

Parameter	Sugar mill effluent concentration (%)						BIS for irrigation water
	0 (BWW)	20	40	60	80	100	
TDS (mg L ⁻¹)	198.50	1,450.00	2,942.00	4,364.00	5,668.00	6,182.00	1,900
Turbidity (NTU)	4.46	1,163	16.58	23.47	29.87	33.42	10
EC (dS cm ⁻¹)	0.34	2.28	4.56	6.79	8.88	9.79	– ^a
pH	7.50	7.78	7.87	7.98	8.82	8.98	5.5–9.0
DO (mg L ⁻¹)	8.24	4.33	3.62	2.44	2.11	NIL	–
BOD (mg L ⁻¹)	3.83	180.36	366.89	775.68	1,230.96	1,632.58	100
COD (mg L ⁻¹)	5.88	234.68	572.45	1,136.78	1,698.78	2,268.96	250
Cl ⁻ (mg L ⁻¹)	15.68	140.52	335.84	652.47	944.58	1,248.82	500
HCO ₃ ⁻ (mg L ⁻¹)	282.00	310.15	332.14	375.48	510.23	662.14	–
CO ₃ ²⁻ (mg L ⁻¹)	105.75	132.47	170.85	189.69	214.36	248.59	–
Na ⁺ (mg L ⁻¹)	9.65	34.36	80.25	138.71	210.58	280.96	–
K ⁺ (mg L ⁻¹)	5.54	40.58	98.67	182.47	268.37	349.82	–
Ca ²⁺ (mg L ⁻¹)	23.46	110.26	248.93	444.45	652.39	842.35	200
Mg ²⁺ (mg L ⁻¹)	12.15	32.64	68.46	102.58	148.96	194.64	–
TKN (mg L ⁻¹)	24.27	42.54	63.88	94.37	110.24	141.24	100
NO ₃ ²⁻ (mg L ⁻¹)	25.17	104.74	236.55	422.87	495.64	788.69	100
PO ₄ ³⁻ (mg L ⁻¹)	0.04	32.44	73.69	146.57	219.78	296.39	–
SO ₄ ²⁻ (mg L ⁻¹)	17.64	114.12	252.36	464.85	684.36	892.87	1,000
Fe ²⁺ (mg L ⁻¹)	0.28	7.36	14.86	21.48	28.44	32.69	1.0
Zn (mg L ⁻¹)	0.06	4.79	9.63	13.69	18.64	20.36	15
Cd (mg L ⁻¹)	0.01	1.86	3.12	5.24	6.48	8.36	2.00
Cu (mg L ⁻¹)	0.04	1.55	2.98	3.99	5.78	6.96	3.00
Mn (mg L ⁻¹)	0.02	3.45	6.98	8.25	10.22	12.48	1.00
Cr (mg L ⁻¹)	0.01	0.18	0.39	1.21	1.42	1.66	2.00
SPC (SPC ml ⁻¹)	4.8 × 10 ³	5.77 × 10 ⁶	4.96 × 10 ⁸	7.66 × 10 ¹⁰	6.87 × 10 ¹²	8.58 × 10 ¹⁴	10,000
MPN (MPN 100 ml ⁻¹)	2.4 × 10 ²	4.46 × 10 ⁴	5.75 × 10 ⁶	6.85 × 10 ⁸	7.77 × 10 ¹⁰	6.69 × 10 ¹²	5,000

BWW well water control, BIS Bureau of Indian standard

^a “–” = Not given in standard

Table 2 ANOVA for effect of sugar mill effluent on soil characteristics

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

SME sugar mill effluent

ns, *, ** Non-significant or significant at $P \leq 0.05$ or $P \leq 0.01$, ANOVA

alkalis used in the sugar manufacturing process. The values of BOD, COD, Cl⁻, Ca²⁺, Fe²⁺, TKN, SO₄²⁻, MPN, and SPC were recorded above the prescribed limits of the Indian Irrigation Standards (BIS 1991). Higher values of BOD and COD might be due to the presence of high oxidizable organic matter and rapid consumption of dissolved

Table 3 ANOVA for effect of sugar mill effluent on concentrations of cations in soil

Source	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺	Fe ²⁺
Season (S)	*	*	*	*	*
SME concentration (C)	**	*	*	*	**
Interaction S × C	**	**	**	**	**

SME sugar mill effluent

*, ** Significant at $P \leq 0.05$ or $P \leq 0.01$

inorganic materials. The higher bacterial load (SPC and MPN) in sugar mill effluent might be due to the presence of more dissolved solids and organic matter in sugar mill effluent as earlier reported by Kumar and Chopra (2010). The values of TKN, PO₄³⁻, K⁺, Ca²⁺, and Mg²⁺ in the effluent were higher than the prescribed standards. In the

Table 4 ANOVA for effect of sugar mill effluent on concentrations of TKN and anions in soil

Source	TKN	PO ₄ ³⁻	SO ₄ ²⁻
Season (S)	*	*	*
SME concentration (C)	**	**	**
Interaction S × C	**	**	**

SME sugar mill effluent

*, ** Significant at $P \leq 0.05$ or $P \leq 0.01$, ANOVA

Table 5 ANOVA for effect of sugar mill effluent on concentrations of metals in soil

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

SME sugar mill effluent

*, ** Significant at $P \leq 0.05$ or $P \leq 0.01$, ANOVA

Table 6 Effects of sugar mill effluent concentration and season interaction on physico-chemical characteristics of soil used in the cultivation of *P. glaucum* in both seasons

SME sugar mill effluent
ns, *, ** Non-significant or significant at $P \leq 0.05$ or $P \leq 0.01$, respectively, Least squares means

Season × % SME	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	1.92	7.63	0.52	25.48	164.53	23.43
	20	2.98ns	7.82ns	3.68*	33.96*	188.55ns	36.86*
	40	5.66*	8.01ns	6.34**	60.85*	237.94**	57.96**
	60	7.78*	8.26*	10.92**	77.86*	265.68**	70.36**
	80	9.86*	8.43*	15.88**	87.63**	290.98*	72.82*
	100	11.72**	8.63*	17.76**	94.36**	313.64*	86.39*
Summer	0	1.94	7.64	0.51	25.68	165.88	23.69
	20	3.72ns	7.94ns	4.01*	35.69*	192.86ns	47.85*
	40	6.36*	8.15ns	6.96**	60.25*	243.68**	70.32**
	60	8.48*	8.37*	11.86**	81.36*	276.98*	77.69**
	80	10.55*	8.52*	16.78**	89.69**	299.36*	84.55*
	100	12.89**	8.72*	19.86**	97.47**	327.45*	98.69*

Table 7 Effects of sugar mill effluent concentration and season interaction on physico-chemical characteristics of soil used in the cultivation of *P. glaucum* in both seasons

Season × % SME	TKN (mg kg ⁻¹)	PO ₄ ³⁻ (mg kg ⁻¹)	SO ₄ ²⁻ (mg kg ⁻¹)	Fe ²⁺ (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Cd (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Cr (mg kg ⁻¹)
Rainy									
0	36.88	63.69	84.58	4.58	0.56	0.33	1.14	0.59	0.29
20	82.36**	98.47*	131.36*	8.86ns	2.96ns	1.22ns	4.40ns	3.05ns	0.29ns
40	174.33**	145.64*	156.78**	19.96*	4.30*	1.56*	6.68*	6.09ns	0.74*
60	295.29**	176.93**	186.68**	38.78*	6.87*	1.94*	9.36**	8.15ns	0.99*
80	358.75**	183.3**	254.36**	58.47**	8.96**	2.11**	12.36**	10.25**	1.58*
100	436.56*	246.78**	286.90**	74.33**	10.12**	2.87**	14.23**	14.86*	1.84**
Summer									
0	37.45	64.24	84.88	4.60	0.57	0.34	1.16	0.62	0.32
20	86.49**	96.79*	139.69*	9.36ns	3.29ns	1.34ns	4.76ns	3.36ns	0.45ns
40	182.88**	143.85*	177.84**	23.25*	4.64*	1.78*	7.81*	6.84ns	0.84*
60	324.24**	184.25**	196.55**	45.63*	7.49*	2.06*	10.15**	9.63ns	1.86*
80	398.96**	193.8**	268.41**	67.75**	9.76**	2.36*	13.48**	12.44**	1.96*
100	457.58**	257.67**	292.37**	78.39**	13.78**	2.98*	15.39**	15.42*	2.14**

SME sugar mill effluents

*, ** Non-significant or significant at $P \leq 0.05$ or $P \leq 0.01$, respectively, Least Squares Means analysis

present study, the contents of BOD, COD, TKN, Cl⁻, SO₄²⁻, and PO₄³⁻ were more in sugar mill effluent than the content of BOD, COD, chlorides, sulfate, and phosphate in sugar mill effluent reported by Ezhilvannan et al. (2011). In the case of metals, the contents of Fe²⁺, Zn, Cd, Cu, Cr, and Mn were higher than permissible limits for industrial effluent (BIS 1991). The contents of these metals in sugar mill effluent were noted higher than the content of Zn, Cd, and Cu in the sugar mill effluent reported by Borole and Patil (2004).

Characteristics of soil

Physico-chemical characteristics of the soil changed due to irrigation with sugar mill effluent (Tables 2, 3, 4, 5, 6, 7, 8; Fig. 1). At harvest of *P. glaucum* (90 days after sowing), there was no significant change in the soil texture (loamy sand; 40 % sand: 40 % silt: 20 % clay). Irrigation with 100 % sugar mill effluent had the most increase in EC, OC,

Table 8 Coefficient of correlation (*r*) between sugar mill effluent and soil characteristics in both seasons

Effluent/soil characteristics	Season	<i>r</i> value
Sugar mill effluent versus soil WHC	Rainy	-0.97
	Summer	-0.97
Sugar mill effluent versus soil BD	Rainy	-0.96
	Summer	-0.96
Sugar mill effluent versus soil EC	Rainy	+0.98
	Summer	+0.98
Sugar mill effluent versus soil pH	Rainy	-0.96
	Summer	-0.96
Sugar mill effluent versus soil OC	Rainy	+0.99
	Summer	+0.99
Sugar mill effluent versus soil Na ⁺	Rainy	+0.97
	Summer	+0.97
Sugar mill effluent versus soil K ⁺	Rainy	+0.87
	Summer	+0.87
Sugar mill effluent versus soil Ca ²⁺	Rainy	+0.76
	Summer	+0.76
Sugar mill effluent versus soil Mg ²⁺	Rainy	+0.63
	Summer	+0.63
Sugar mill effluent versus soil TKN	Rainy	+0.99
	Summer	+0.99
Sugar mill effluent versus soil PO ₄ ³⁻	Rainy	+0.99
	Summer	+0.99
Sugar mill effluent versus soil SO ₄ ²⁻	Rainy	+0.96
	Summer	+0.96
Sugar mill effluent versus soil Fe ²⁺	Rainy	+0.99
	Summer	+0.99
Sugar mill effluent versus soil Zn	Rainy	+0.94
	Summer	+0.94
Sugar mill effluent versus soil Cd	Rainy	+0.97
	Summer	+0.97
Sugar mill effluent versus soil Cu	Rainy	+0.99
	Summer	+0.99
Sugar mill effluent versus soil Mn	Rainy	+0.99
	Summer	+0.99
Sugar mill effluent versus soil Cr	Rainy	+0.99
	Summer	+0.99

Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe²⁺, TKN, PO₄³⁻, SO₄²⁻, Cd, Cr, Cu, Mn, and Zn in both seasons (Tables 6, 7). The values of WHC and BD were insignificantly changed by the different concentrations of sugar mill effluent in both the cultivated seasons. WHC and BD were reduced from their initial (control) values 45.58 % and 1.42 gm cm⁻³ to 42.13, 41.36 %, and 1.41 gm cm⁻³, respectively, with 100 % sugar mill effluent concentration. Season, sugar mill effluent concentration, and interaction of seasons and sugar mill effluent concentration did not affect the WHC and BD (Table 2). WHC is related to the number and size

Table 10 ANOVA for effect of sugar mill effluent on maturity stage of *P. glaucum*

Source	Chlorophyll content	LAI	Spikes length	CY/plant	HI
Season (S)	ns	ns	ns	ns	ns
SME concentration (C)	*	*	*	*	ns
Interaction S × C	*	*	*	*	ns

ns non-significant, SME sugar mill effluent

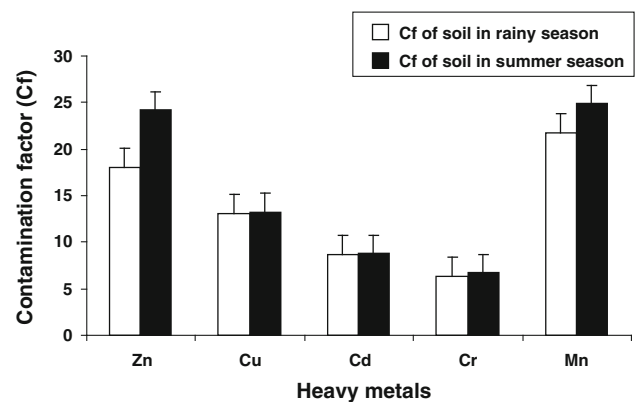


Fig. 1 Contamination factor of heavy metals in soil after irrigation with sugar mill effluent. Error bars are standard error of the mean

Table 9 ANOVA for effect of sugar mill effluent on germination and vegetative growth of *P. glaucum*

Source	Seed germination	Relative toxicity	Plant height	Root length	No. of tillers	No. of leaves	Fresh weight	Dry weight
Season (S)	ns	ns	ns	ns	ns	ns	ns	ns
SME concentration (C)	*	ns	*	ns	ns	ns	*	ns
Interaction S × C	*	ns	*	ns	ns	ns	*	ns

SME sugar mill effluent

ns, *, Non-significant or significant at $P \leq 0.05$, ANOVA

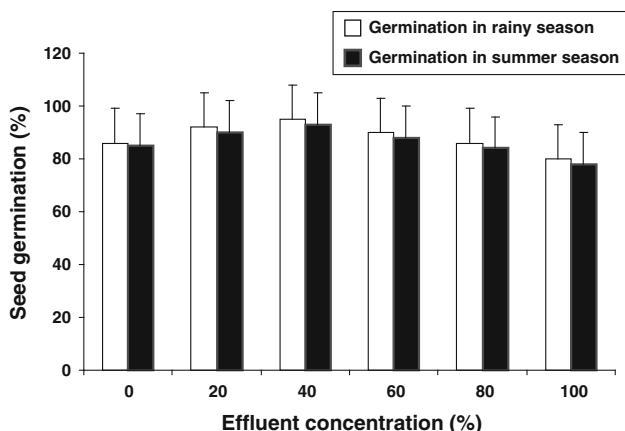


Fig. 2 Seed germination of *P. glaucum* after irrigation with sugar mill effluent. Error bars are standard error of the mean

distribution of soil pores, soil moisture content, textural class, structure, salt content, and organic matter. The BD of soil changes with the application of organic manure to soil that substantially modifies, and lowers the soil bulk density. It is used for determining the amount of pore space and water storage capacity of the soil. Organic matter supplied through the sugar mill effluent and other kind of wastes, like sludge, can lower the BD and WHC (Roy et al. 2007). The findings were also in accordance to Baskaran et al. (2009). Fertigation with 100 % sugar mill effluent concentration increased pH (13.10–14.13 %), EC (510.41–564.43 %), OC (3,238.46–3794.11 %), Na⁺ (270.32–279.55 %), K⁺ (90.44–97.40 %), Ca²⁺ (748.97–778.33 %), Mg²⁺ (268.71–316.58 %), TKN (1,083.73–1,121.84 %), PO₄³⁻ (287.47–301.10 %), SO₄²⁻ (239.20–244.45 %), Fe²⁺ (1,522.92–1,604.13 %), Cd (769.69–776.47 %), Cr (534.48–568.75 %), Cu (1,212.28–1226.72 %), Mn (2,079.66–2,387.09 %), and Zn (1,707.14–2,317.54 %) in the soil in both seasons.

Sugar mill effluent concentration affected pH and EC of the soil but not season. Season, sugar mill effluent concentration and their interaction affected OC, and TKN of the soil (Tables 2, 4). The 40–100 % sugar mill effluent concentration affected EC, OC, Na⁺, K⁺, Ca²⁺, Mg²⁺, TKN, Fe²⁺, PO₄³⁻, SO₄²⁻, Cr, Cu, and Zn in *P. glaucum* cultivated soil in both seasons. The 20 % sugar mill effluent concentration also affected OC, Na⁺, Ca²⁺, Mg²⁺, TKN, PO₄³⁻, and SO₄²⁻ in both seasons (Tables 6, 7). Soil

pH was affected by the 60, 80, and 100 % sugar mill effluent concentrations while Mn was affected by the 80 and 100 % sugar mill effluent concentrations (Table 6). The EC, OC, Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe²⁺, TKN, PO₄³⁻, SO₄²⁻, Cd, Cr, Cu, Mn, and Zn positively correlated with sugar mill effluent concentration in both seasons (Table 8). In the present study, more irrigation of *P. glaucum* with sugar mill effluent considerably increased the content of OC, Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe²⁺, TKN, PO₄³⁻, SO₄²⁻, Cd, Cr, Cu, Mn, and Zn in soil. Season, sugar mill effluent concentration and the their interaction affected all cations like Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe²⁺ and anions PO₄³⁻ and SO₄²⁻ of the soil (Tables 3, 4).

Baskaran et al. (2009) reported that sugar mill effluent increased EC, pH, total organic carbon (TOC), total Kjeldahl nitrogen (TKN), and available phosphorus, exchangeable Na, K, Ca and Mg in soil. Effluent irrigation generally adds PO₄³⁻, HCO₃⁻, Cl⁻, Na⁺, Ca²⁺, K⁺, Mg²⁺, Cd, Cr, Cu, Ni, Mn, and Zn to the soil (Chopra et al. 2009).

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 distillery effluent to be higher than in the soil irrigated with bore well water. Average values of TKN, PO₄³⁻, 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, PO₄³⁻, and K⁺ in the soil was due to irrigation with TKN, PO₄³⁻, and K⁺ rich sugar mill effluent. The content of Na⁺ and SO₄²⁻ was higher in the soil irrigated with sugar mill effluent indicating a link between soil Na⁺ and SO₄²⁻ and higher EC in the sugar mill effluent.

The contents of heavy metals, Cd, Cr, Cu, Mn, and Zn, in the soil increased as the effluent concentration increased (Table 7). Season, sugar mill effluent concentration, and their interaction affected all metals, Cd, Cr, Cu, Mn, and Zn in soil (Table 5). The concentration of Mn was found maximum while that of Cr was low after sugar mill effluent irrigation in both seasons. The contamination factor (Cf) of the heavy metals indicated that Mn was the highest while Cr was lower in both seasons after irrigation with 100 % sugar mill effluent. The Cf of heavy metals were in the

Table 11 ANOVA for effect of sugar mill effluent on concentrations of metals in *P. glaucum*

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

SME sugar mill effluent

ns, *, ** Non-significant or significant at $P \leq 0.05$ or $P \leq 0.01$, ANOVA

order of Mn > Zn > Cu > Cd > Cr after irrigation with sugar mill effluent in both seasons (Fig. 1). The concentration of heavy metals Cd, Cr, Cu, Mn, and Zn was higher in soil irrigated with effluent than in soil irrigated with control water. Thus, fertigation with sugar mill effluent increased nutrients as well as metals content in soil.

Effect on germination

At 0–15 days after sowing, the best germination (95 and 93 %) was for with 40 % sugar mill effluent and the least (80 and 78 %) was due to treatment with 100 % sugar mill effluent (Fig. 2). Germination of *P. glaucum* was negatively correlated with sugar mill effluent concentrations in both seasons (Table 11). Seed germination of *P. glaucum* was affected by treatment (Table 9). The ANOVA indicated that season had no effect on plant germination and relative toxicity. Sugar mill effluent concentration and their

interaction with season affected plant germination of *P. glaucum*, but not relative toxicity (Table 9).

The maximum RT (108.97 and 107.50 %) of sugar mill effluent against germination of *P. glaucum* was for the 100 % sugar mill effluent and it was positively correlated with sugar mill effluent concentrations in both seasons (Table 11, Fig. 3). The findings were very much in accordance with Radhouane (2008) reported that the germination of millet cultivars was decreased as concentration of the waste effluent increased from 0 to 100 %.

In the present investigation, the higher concentration of sugar mill effluent did not support plant germination. The higher concentration of sugar mill effluent lowered germination of *P. glaucum* likely due to the presence of high salt content in the effluent at these concentrations which inhibit germination. High concentrations are usually most damaging to young plants but not necessarily at germination, although the 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 delaying germination (Sunseri et al. 1998; Kaushik et al. 2004).

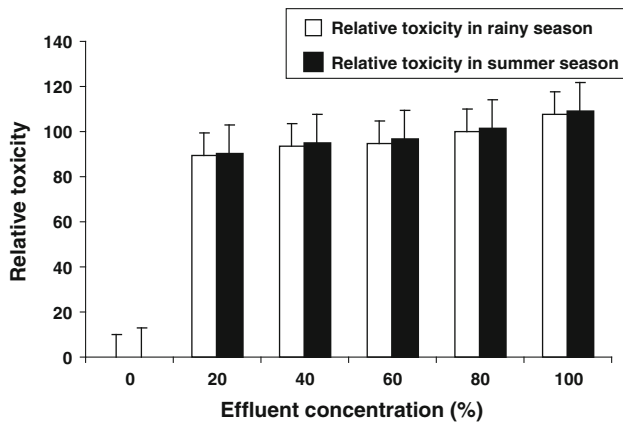


Fig. 3 Relative toxicity of sugar mill effluent against seed germination of *P. glaucum*. Error bars are standard error of the mean

Effect on vegetative growth stage

Vegetative growth of *P. glaucum* at 45 days was affected in both seasons (Tables 9, 12). Maximum plant height, fresh weight, chlorophyll content, and LAI/plant of *P. glaucum* were due to treatment with the 40 % concentration of sugar mill effluent in both seasons (Table 12). Average root length (14.55 and 16.75 cm), number of tillers (4.67 and 5.96), number of leaves (14.88 and 16.36), and dry weight/plant (54.19 and 56.65 g) of *P. glaucum* were in control while root length (12.44 and 13.63 cm), number of tillers (3.21

Table 12 Effects of sugar mill concentration and season interaction on agronomical parameters of *P. glaucum*

Season × % SME	Plant height (cm)	Fresh weight (g)	Chlorophyll content (mg (g f wt) ⁻¹)	LAI	Spike length (cm) Kernels	Crop yield/plant (g)
Rainy						
0	216.54	123.13	3.32	2.35	16.58	32.42
20	255.82*	143.56*	3.95ns	3.55ns	18.63ns	44.75ns
40	314.29*	165.34*	4.81*	5.19*	28.58*	50.59*
60	265.84*	152.98*	4.71*	4.76*	25.36*	46.32*
80	234.78*	142.34*	4.42*	4.43*	22.48*	40.67 ns
100	225.36*	130.56 ns	3.45 ns	3.81 ns	20.18 ns	38.88 ns
Summer						
0	208.47	115.34	3.12	2.12	14.46	30.99
20	242.45*	137.56*	3.46ns	3.46ns	16.63ns	42.87ns
40	301.23*	158.76*	4.62*	5.12*	27.44*	48.89*
60	255.48*	147.23*	4.29*	4.29*	24.66*	42.98ns
80	225.32*	135.55*	4.18*	4.37*	20.75*	38.78ns
100	217.88*	124.78ns	3.38ns	3.68ns	18.56ns	36.89ns

SME sugar mill effluent
 * Significant at P ≤ 0.05, Least Means Squares analysis

and 3.55), number of leaves (12.55 and 13.75), and dry weight/plant (43.89 and 44.38 g) of *P. glaucum* were with 100 % sugar mill effluent in both seasons. The maximum root length (18.36 and 20.36 cm), number of tillers (7.45 and 7.89), number of leaves (18.87 and 18.96), and dry weight/plant (61.36 and 62.87 g) of *P. glaucum* were with 40 % concentration of sugar mill effluent in both seasons. Sugar mill effluent concentration, season, and their interaction had no effect on root length, number of tillers, number of leaves, and dry weight of *P. glaucum* (Tables 9, 10).

The ANOVA indicated that the sugar mill effluent concentration affected plant height, fresh weight, chlorophyll content, and LAI/plant of *P. glaucum* (Tables 9, 10). The season had no effect on plant height, fresh weight, chlorophyll content, and LAI/plant of *P. glaucum*. The interaction of the season and sugar mill effluent concentrations affected plant height, fresh weight, chlorophyll content, and LAI/plant of *P. glaucum* (Tables 9, 10).

Plant height, number of tillers, number of leaves, fresh weight, dry weight, chlorophyll content, and LAI/plant of *P. glaucum* were positively correlated with sugar mill effluent concentrations in both seasons (Table 11). Root length was positively correlated with sugar mill effluent concentrations in the rainy season while it was negatively correlated in the summer season (Table 11). The findings were in accordance with Moazzam et al. (2012).

Vegetative growth of *P. glaucum* was lowered at higher concentrations of sugar mill effluent. A high EC indicates higher salt content in the higher sugar mill effluent concentrations, which lowered the plant height, root length, number of tillers, number of leaves, fresh weight, dry weight, chlorophyll content, and LAI/plant of *P. glaucum*. Vegetative growth is associated with the development of new shoots, twigs, leaves, and leaf area. Plant height, fresh weight, chlorophyll content, and LAI/plant of *P. glaucum* were higher at 40 % of sugar 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 (Al-Tahir et al. 1997). It is also involved in cell enlargement and in triggering young tissue or meristematic growth (Arya et al. 1997; Radhouane 2008). Chlorophyll content was higher due to the use of 40 % sugar mill effluent in both seasons, and is likely due to Fe, Mg, and Mn contents in the sugar mill effluent, which are associated with chlorophyll synthesis (Porra 2002). The 40 % sugar mill effluent concentration contains optimum contents of nutrients required for maximum vegetative growth of *P. glaucum*.

Effect on maturity stage

On maturity stage (90 days after sowing), the spikes length and crop yield/plant of *P. glaucum* was with 40 % sugar

mill effluent in both seasons (Table 12). Spikes length and crop yield of *P. glaucum* decreased as the sugar mill effluent concentration decreased (Table 12). Maximum HI (156.04 and 157.76 %) was with 40 % sugar mill effluent concentration and (119.03 and 119.92 %) with 100 % sugar mill effluent in both seasons. Sugar mill effluent concentration affected spikes length and crop yield of *P. glaucum* but season, interaction of the season and sugar mill effluent concentration did not have an effect on spikes length, crop yield of *P. glaucum* (Table 10). Season and sugar mill effluent concentration had no effect on Harvest

Table 13 Coefficient of correlation (r) between sugar mill effluent and *P. glaucum* in both seasons

Effluent/French bean	Season	r value
Sugar mill effluent versus seed germination	Rainy	-0.53
	Summer	-0.59
Sugar mill effluent versus RT	Rainy	+0.76
	Summer	+0.76
Sugar mill effluent versus plant height	Rainy	+0.10
	Summer	+0.07
Sugar mill effluent versus root length	Rainy	+0.13
	Summer	-0.14
Sugar mill effluent versus number of tillers	Rainy	+0.56
	Summer	+0.48
Sugar mill effluent versus number of leaves	Rainy	+0.58
	Summer	+0.41
Sugar mill effluent versus fresh weight	Rainy	+0.07
	Summer	+0.10
Sugar mill effluent versus dry weight	Rainy	+0.05
	Summer	+0.03
Sugar mill effluent versus chlorophyll content	Rainy	+0.16
	Summer	+0.28
Sugar mill effluent versus LAI	Rainy	+0.50
	Summer	+0.50
Sugar mill effluent versus spikes length	Rainy	+0.32
	Summer	+0.33
Sugar mill effluent versus crop yield/plant	Rainy	+0.05
	Summer	-0.15
Sugar mill effluent versus HI	Rainy	+0.10
	Summer	+0.09
Sugar mill effluent versus Zn	Rainy	+0.95
	Summer	+0.97
Sugar mill effluent versus Cd	Rainy	+0.86
	Summer	+0.87
Sugar mill effluent versus Cu	Rainy	+0.99
	Summer	+0.99
Sugar mill effluent versus Mn	Rainy	+0.99
	Summer	+0.98
Sugar mill effluent versus Cr	Rainy	+0.89
	Summer	+0.90

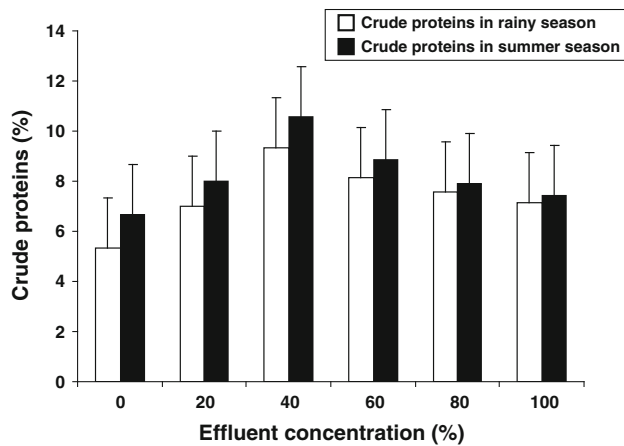


Fig. 4 Crude proteins in *P. glaucum* after irrigation with sugar mill effluent. Error bars are standard error of the mean

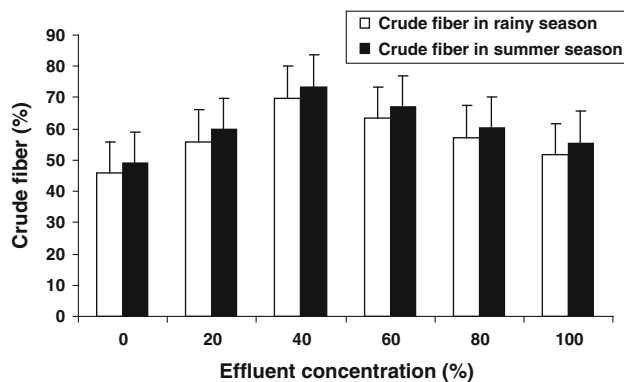


Fig. 5 Crude fiber in *P. glaucum* after irrigation with sugar mill effluent. Error bars are standard error of the mean

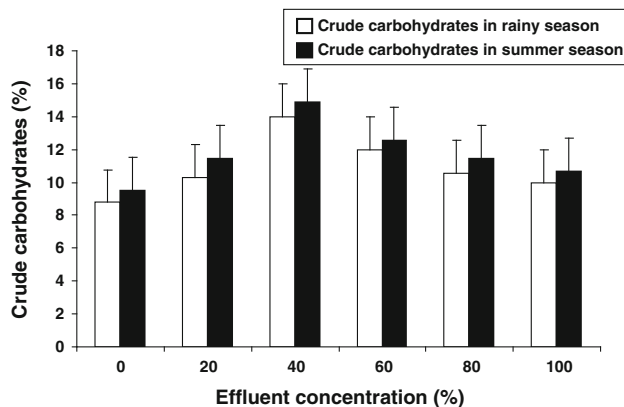


Fig. 6 Crude carbohydrates in *P. glaucum* after irrigation with sugar mill effluent. Error bars are standard error of the mean

index (HI) of *P. glaucum*. Crop yield of *P. glaucum* was positively correlated with sugar mill effluent concentrations in the rainy season while it was negatively correlated in the summer season (Table 11). The results were supported by Zalawadia and Raman (1994).

Nitrogen and phosphorus are essential for flowering and spike length. Too much nitrogen can delay, or prevent, flowering while phosphorus deficiency is sometimes associated with poor flower production, or flower abortion. Maximum spike length of *P. glaucum* was with the 40 % sugar mill effluent; it might be due to that this concentration contains sufficient nitrogen and phosphorus. Furthermore, P and K prevent flower abortion, so grain formation occurs (El-Naggar 2005). Spikes length of *P. glaucum* was lower at higher concentrations of sugar 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 sugar mill effluent concentrations (Pandey et al. 2008). The role of K, Fe, Mg, and Mn at maturity is important and associated with synthesis of chlorophyll, and enhances the formation of grains at harvest (El-Naggar 2005; Naem et al. 2006). The K, Fe, Mg, and Mn contents could benefit grain filling and yield as it does for pearl millet (*P. glaucum* L.) as reported by Moazzam et al. (2012). The 40 % sugar mill effluent favored grains formation and crop yield of *P. glaucum*. This is likely due to the presence of K, Fe, Mg, and Mn contents in 40 % sugar mill effluent; higher sugar mill effluent concentrations lowered grains formation and crop yield of *P. glaucum*.

Effect on biochemical constituents and micronutrients in *P. glaucum*

Season, sugar mill effluent concentration, and the interaction of the season and sugar mill effluent concentration affected all the metals like crude proteins, crude fiber, and crude carbohydrates, Cd, Cr, Cu, Mn and Zn in *P. glaucum* (Table 13). Maximum crude proteins, crude fiber, and crude carbohydrates were recorded with 40 % sugar mill effluent concentrations in both seasons (Figs. 4, 5, 6). Content of crude proteins ($r = + 0.38$), crude fiber ($r = + 0.17$) and crude carbohydrates ($r = + 0.13$) was noted positively correlated with sugar mill effluent concentration in both seasons. The 20, 40, 60, 80, and 100 % sugar mill effluent concentrations affected Cd, Cr, Cu, Mn, and Zn contents in *P. glaucum* (Table 13). Increased irrigation frequency could lead to increases of metals in tissues. The Cd, Cr, Cu, Mn, and Zn contents in *P. glaucum* was the highest with 100 % sugar mill effluent (Figs. 7, 8). They were positively correlated with content of Cd, Cr, Cu, Mn, and Zn in *P. glaucum* after irrigation with sugar mill effluent concentrations in both seasons (Table 11). The contamination factor (Cf) was affected in both seasons (Fig. 9). The Cf of various heavy metals was in the order of Mn > Zn > Cu > Cr > Cd in *P. glaucum* after irrigation with sugar mill effluent (Fig. 9). The highest contamination factor was for Mn; the least was for Cd in *P. glaucum* with 100 % sugar mill effluent in both seasons. The micronutrient contents were higher at higher sugar mill



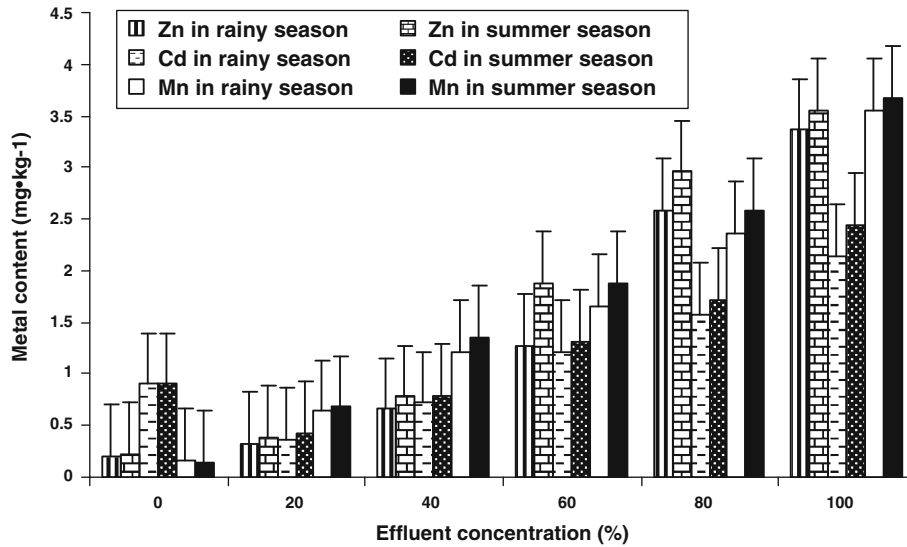


Fig. 7 Content of Zn, Cd and Mn in *P. glaucum* after irrigation with sugar mill effluent. Error bars are standard error of the mean

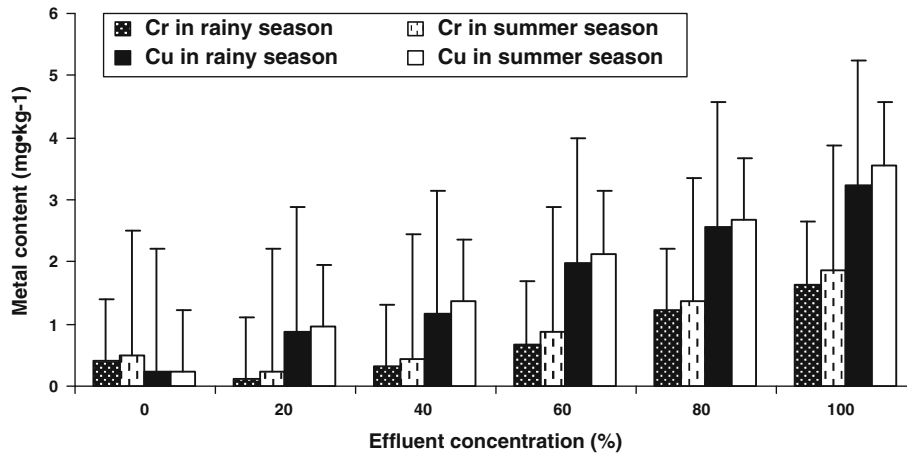


Fig. 8 Content of Cr and Cu in *P. glaucum* after irrigation with sugar mill effluent. Error bars are standard error of the mean

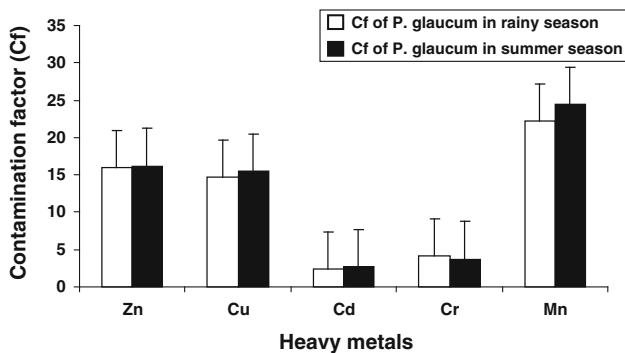


Fig. 9 Contamination factor of heavy metals in *P. glaucum* after irrigation with sugar mill effluent. Error bars are standard error of the mean

effluent concentration, and likely inhibited growth of *P. glaucum*. The 40 % sugar mill effluent favored vegetative growth, flowering, and maturity of *P. glaucum*. This is likely due to optimal uptake of these micronutrients by crop plants, which supports various biochemical and physiological processes.

Conclusions

The present study concluded that the sugar mill effluent increased nutrients in the soil and affected the agronomical characteristics of *P. glaucum* in both seasons. The maximum agronomical performance of *P. glaucum* was observed with 40 % concentration of the sugar mill

effluent. It appears that sugar mill effluent can be used as a biofertilizer after appropriate dilution to improve yield of this crop. Further studies on the agronomic growth and changes in biochemical composition of *P. glaucum* after sugar mill effluent irrigation are required.

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