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Full Length Research Paper

Fertigational impact of sugar mill effluent on agronomical practices of Mung bean (*Vigna radiata* L.) in two seasons

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The present study was aimed to assess the agro-potentiality of sugar mill effluent (SME) as agro-based organic bio-fertigant. The fertigation response of 5%, 10%, 25%, 50%, 75% and 100% of SME doses on *Vigna radiata* L., cv. Pusa-105 in the rainy and summer seasons was investigated. The fertigant concentrations produced significant ($P<0.01$) changes in the electrical conductivity (EC), pH, organic carbon (OC), sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), total Kjeldhal nitrogen (TKN), phosphate (PO_4^{3-}), sulphate (SO_4^{2-}), iron (Fe^{2+}), cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn) and zinc (Zn) of the soil in both seasons. The most shoot, root length, dry weight, chlorophyll content, leaf area index (LAI), number of flowers, pods, crop yield, harvest index (HI), crude proteins, crude fiber and total carbohydrates of *V. radiata* was noted with 25% concentration of the SME in both seasons compared to the controls. The accumulation of metals was increased in the soil and *V. radiata* increased from 5% to 100% concentrations of the SME. The contamination factor (Cf) of various metals was observed in the order of Cr>Cd>Mn>Zn>Cu for soil and Mn>Zn>Cu>Cr>Cd for *V. radiata* in both seasons after fertigation with SME. The contents of Cu, Mn and Zn except Cd and Cr in the soil and *V. radiata* were noted under the permissible limit of Indian soil standards and FAO/WHO standards respectively. Therefore, SME has the potentiality as agro-based bio-fertigant and can be used to improve the soil fertility and yield of *V. radiata* after appropriate dilution.

Keywords: Sugar mill effluent, fertigation, *Vigna radiata*, heavy metals, contamination factor, rainy season, summer season.

INTRODUCTION

Organic farming is emerging as a sustainable alternative in reviving agriculture especially in areas where the indiscriminate usage of chemical fertilizers and pesticides had resulted in loss in soil fertility and productivity with adverse effects on water quality, soil, plant and human health (Ezhilvannan et al., 2011; Buvaneswari et al., 2013; Kumar and Chopra, 2014). The reuse of effluent by irrigation can make a significant contribution to the integrated management of our water resources. It is an opportune time to refocus on one of the ways to recycle water through the reuse of industrial and urban wastewater, for irrigation and other purposes (Kumar and Chopra, 2010; Vijayaragavan et al., 2011). Thus, the value of wastewater for crop production has been recognized in many countries, including India (Vijayaragavan et al., 2011; Buvaneswari et al., 2013). The effluents not only contain nutrients that stimulate

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ABBREVIATIONS

ANOVA - Analysis of variance, **BD** - Bulk density, **BIS** - Bureau of Indian Standards, **BWW** - Bore well water, **HI** - Harvest index, **LAI** - Leaf area index, **MPN** - Most probable numbers, **RT** - Relative toxicity, **SME** – Sugar mill effluent, **SPC** - Standard plate count, **WHC** - Water holding capacity.

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 (Kumar and Chopra, 2012, 2013a). Irrigation and fertilizers are two main important inputs which are required to attain maximum crop yield. The reuse of industrial effluent is the need of time as the water level is going down rapidly in the country. The utilization of industrial effluents as soil amendment and fertigation of crops has generated interest in recent times. Most crops give higher potential yields with wastewater fertigation, and reduce the need for chemical fertilizers, resulting in net cost savings to farmers (Kumar and Chopra, 2012, 2014).

Additionally, environmental pollution has been documented as one of the major problems of the modern world (Khan et al., 2003; Maliwal et al., 2004; Buvaneswari et al., 2013). The problem of water pollution is due to the generation of huge volume of the effluent and their disposal (Saranraj and Stella, 2012; Khan et al., 2003). India is one of the largest producers of sugar in the world (Buvaneswari et al., 2013; Ezhilvannan et al., 2011). There are about 650 sugar mills which are potentially producing about 182 lakh tonnes of sugar per year (Kumar and Chopra, 2010). The sugar industry is playing an important role in the economic development of the Indian subcontinent, but the effluents released produce a high degree of organic pollution in both the aquatic and terrestrial ecosystems (Ramakrishnan et al., 2001; Swamy et al., 2001; Vijayaragavan et al., 2011). The sugar industry is one of the most important agro based industries in India and is highly responsible for creating significant impact on the rural economy in particular and the countries economy in general (Lakshmi and Sundaramoorthy, 2002; Maliwal et al., 2004). Sugar industries rank second among the agro based industries in India (Sanjay and Solomon, 2005; Roy et al., 2007).

The sugar mill effluent is mainly discharged from crushing, refining, mill house, boiling house, and condensate water (Saranraj and Stella, 2012; Khan et al., 2003). The disposal of effluent is one of the main problems of today and has to be faced in the future with its increased adverse effects (Krishna and Leelavathi, 2002; Amathussalam et al., 2002 Kumar and Chopra, 2010; Vijayaragavan et al., 2011). Most of the sugar mills are discharging their effluent into the environment without any treatment or partial treatment (Kisku et al., 2000; Rathore et al., 2000; Khan et al., 2003). The effluent when discharged into the environment, poses a serious health hazard to the rural and semi-urban populations that uses the stream and river water for agriculture and domestic purposes (Barman et al., 2000; Ayyasamy et al., 2008; Vijayaragavan et al., 2011; Kumar and Chopra, 2014).

Furthermore, sugar mills play a major role in polluting the aquatic and terrestrial environment by discharging a huge amount of wastewater as effluent (Samuel and Muthukkaruppan, 2011; Kumar and Chopra, 2013a, 2014). The sugar mill effluents are having a higher amount of total dissolved solids (TDS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), chlorides (Cl^-) SO_4^{2-}, nitrates (NO_3^{2-}), Ca^{2+} and Mg^{2+}, etc (Buvaneswari et al., 2013; Ezhilvannan et al., 2011). In addition to that, some heavy metals such as Cu, Fe, lead (Pb), Mn and Zn are usually present in the effluent (Ezhilvannan et al., 2011; Borole et al., 2004; Roy et al., 2007).

The use of untreated effluents harmfully affects the soil fertility and agricultural crops when used for fertigation (Hati et al., 2007; Samuel and Muthukkaruppan, 2011; Saranraj and Stella, 2012). Thus, excess amount of salts and heavy metals get accumulated in the soil and crops and make them polluted (Chandrasekar et al., 1998; Kisku et al., 2000; Khan et al., 2003; Kaushik et al., 2004). Besides this use of sugar mill effluent as organic fertilizer and soil amendment, it has become popular in the agriculture (Baskaran et al., 2009; Ezhilvannan et al., 2011; Vijayaragavan et al., 2011). Effluents also contain appreciable amounts of nutrients and metals like Cd, Cu, Fe, Pb, Mn, Ni and Zn (Bharagava et al., 2008; Biswas et al., 2009; Ezhilvannan et al., 2011; Buvaneswari et al., 2013). Irrigation with such effluent increases nitrogen, phosphorus, potassium, organic carbon and heavy metal contents in the soil and crops (Hati et al., 2007; Chopra et al., 2012; Kumar and Chopra, 2013b, 2014).

Mung bean (*Vigna radiata* L.) is an important pulse and fodder crop cultivated in tropical and subtropical provinces of the world. In India, it is cultivated in rainy and summer seasons (Chandrasekar et al., 1998). Around the world, young, whole beans are eaten as a green vegetable; they are consumed directly and used in various dishes including curries, soups, breads, sweets, noodles and solids. The crop *V. radiata* pacifies general weakness, vertigo, neuropathy, peptic ulcer, indigestion, fever, and diarrhea (Baskaran et al., 2009).

Several crops have higher potential yields with wastewater irrigation; reduce the need for chemical fertilizers, and result in net cost savings to farmers (Ezhilvannan et al., 2011; Kumar and Chopra, 2013a). Therefore, utility potential of industrial effluents for irrigation of crop fields has been a controversial proposition due to the contradictory reports obtained on the effects of these effluents on crop plant responses (Amathussalam et al., 2002; Khan et al., 2003; Roy et al., 2007; Samuel and Muthukkaruppan, 2011). 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; Baskaran et al., 2009; Ezhilvannan et al., 2011; Vijayaragavan et al., 2011; Kumar and Chopra, 2014). However, no detailed experiments have been performed on the plant growth and biochemical changes using sugar mill effluent (Baskaran et al., 2009). The aim of the present investigation was to study the agro-potentiality of sugar mill effluent as an agro-based organic biofertigant on agronomical practices of *Vigna radiata* (L.) in two seasons.

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 agro-potentiality of sugar mill effluent (SME) fertigation on *V. radiata.* Seven plots (each plot had an area of 9 m^2) were selected for seven treatments of SME namely: 0% (control), 5%, 10%, 25%, 50%, 75% and 100% for the cultivation of *V. radiata.* The treatments were placed within randomized complete block design.

Effluent collection and analysis

The effluent samples were collected from the R.B.N.S. Sugar mill, Laksar, Haridwar, Uttarakhand (29°44'46''N 78°1'46''E), which produces sugar from sugar cane at the rate of 150 ton sugar per day and generates the effluent about 2500 m^3/day. The effluent was collected in plastic containers from a settling tank installed in the campus, by the sugar mill to reduce BOD and TDS from the effluent. The samples were brought to the laboratory and analyzed for TDS, pH, EC, dissolved oxygen (DO), BOD, COD, Cl^-, bicarbonates (HCO_3^-), carbonates (CO_3^{2-}), Na^+, K^+, Ca^{2+}, Mg^{2+}, TKN, NO_3^{2-}, PO_4^{3-}, SO_4^{2-}, Fe, Cd, Cr, Cu, Mn, Zn, standard plate count (SPC) and most probable number (MPN) following APHA (2005) and Chaturvedi and Sankar (2006), and used as fertigant.

Sowing of seeds of *V. radiata*

Seeds of *V. radiata* were sown at the end of February 2008 and 2009 for the summer season crop and at the end of August 2008 and 2009 for the rainy season crop. Seeds of *V. radiata*, cv. Pusa-105, 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 20 cm (Kumar, 1997). The thinning (removal of the densely germinated plants) was done manually after 15 days of germination to maintain the desired plant spacing and to avoid competition between plants.

Irrigation pattern, soil sampling and analysis

The soil in each plot was fertigated twice in a month with50 gallons of sugar mill effluent with 5%, 10%, 25%, 50%, 75% and 100% along with bore well water as the control. The soil samples were analyzed prior to planting and after harvest for various physico-chemical parameters namely, bulk density (BD), water holding capacity (WHC), soil texture, soil pH, EC, OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, TKN, PO_4^{3-}, SO_4^{2-}, Fe^{2+}, Zn, Cd, Cu, Mn and Cr determined following standard methods (Chaturvedi and Sankar, 2006).

Study of crop parameters

The agronomic parameters of *V. radiata* at different stages (0-90 days) were determined following standard methods for seed germination, shoot length, root length, number of flowers, number of pods and crop yield (Chandrasekar et al., 1998); dry weight (Milner and Hughes, 1968); chlorophyll content (Porra, 2002); relative toxicity (RT) (Kumar and Chopra, 2013a), Leaf Area Index (LAI) (Denison and Russotti, 1997) and HI (Sinclair, 1998). The nutrients 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 were determined by the anthrone reagent method (Cerning and Guilhot, 1973).

Extraction of heavy metals and their analysis

For heavy metals analysis, a 10 ml sample of SME, and 1.0 g of air dried soil or plants were taken in the digestion tubes separately. For each sample, 3 ml of concentrate HNO_3 was added and digested in an electrically heated block for 1 h at 145°C. To this mixture, 4 ml of $HClO_4$ was added and heated to 240°C for 1 h. The mixture was cooled and filtered through Whatman # 42 filter paper. The volume was made with 50 ml with double distilled water and used for analysis. Heavy metals were analyzed using an atomic absorption spectrophotometer (PerkinElmer, Analyst 800 AAS, GenTech Scientific Inc., Arcade, NY) following methods of APHA (2005) and Chaturvedi and Sankar (2006). The contamination factor (Cf) for heavy metals accumulated in the SME irrigated soil and *V. radiata* was calculated following Håkanson (1980).

Data analysis

Data were analyzed with SPSS (ver. 12.0, SPSS Inc., Chicago, Ill.) and subjected to two-way ANOVA. Duncan's multiple range test was also performed to determine whether 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 were significantly ($P<0.05$) different over the SME concentrations (Table 1). The SME was alkaline, that is, pH 8.77 and it was noted insignificantly ($P>0.05$) different for the concentrations of the SME. The values of BOD, COD, Cl^-, K^+, Ca^{2+}, Fe^{2+}, TKN, PO_4^{3-}, SO_4^{2-}, MPN and SPC in the SME were found above the prescribed limits of the Indian Irrigation Standards (BIS, 1991). Moreover, the SME was rich in organic and inorganic nutrients and heavy metals. The SME showed higher bacterial load in terms of SPC and MPN (Table 1).

In the present study, the pH of SME was alkaline and the alkaline nature of the SME might be due to the presence of high concentration of organic acids in the SME. The higher values of BOD and COD might be due to the presence of high oxidizable organic matter and rapid consumption of dissolved inorganic materials (Tiwari et al., 2000). The higher bacterial load (SPC and MPN) in the SME might be due to the presence of more dissolved solids and organic matter in the effluent as earlier reported by Kumar and Chopra (2010). In the present study, the values of BOD, COD, TDS, Cl^-, Ca^{2+}, Mg^{2+}, SO_4^{2-} and PO_4^{3-} in the SME were more than the content of BOD (1090.00 mgL^{-1}), COD (3260.00 mgL^{-1}), TDS (1540.00 mgL^{-1}), Cl^- (377.00 mgL^{-1}), Ca^{2+} (393 mgL^{-1}), Mg^{2+} (299.00 mgL^{-1}), SO_4^{2-} (430.00 mgL^{-1}) and PO_4^{3-} (6.17 mgL^{-1}) in the sugar mill effluent reported by Saranraj and Stella (2012). The contents of Cd, Cr Cu, Fe^{2+}, Mn and Zn were higher than the permissible limits for industrial effluent (BIS, 1991). The contents of these metals in the sugar mill effluent were lower than the content of Fe (12.80 mgL^{-1}), Zn (0.26 mgL^{-1}), Cd (0.06 mgL^{-1}) and Cu (0.14 mgL^{-1}) in the sugar mill effluent earlier reported by Samuel and Muthukkaruppan (2011).

Characteristics of soil

The physico-chemical characteristics of the soil changed due to fertigation with different concentrations of the SME (Tables 2 to 7). At harvest (90 days after sowing), there was no significant ($P>0.05$) change in the soil texture (loamy sand - 60% sand: 20% silt: 20% clay). Fertigation with 100% concentration of the SME 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 of the soil in both seasons (Tables 5 and 6). The values of WHC and BD were found to be insignificantly ($P>0.05$) different with all the concentrations of the SME in both cultivated seasons (Table 2). WHC and BD were decreased from their initial (control) values of 40.98% and 1.40 gm cm^{-3} to 40.08, 40.25% and 1.39 gm cm^{-3} respectively with 100% concentration of the SME. Seasons, SME concentrations and the their interaction significantly ($P<0.01$) affected the EC, OC, TKN, all the cations Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+} and anions PO_4^{3-} and SO_4^{2-} of the soil (Tables 2 and 3).

In the present study, more irrigation of *V. radiata* significantly ($P<0.01$) increased the contents of OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, TKN, PO_4^{3-}, SO_4^{2-} Zn, Cd, Cu, Mn and Cr in the soil (Tables 5 and 6). The 25% to 100% concentrations of the SME showed significant ($P<0.01$) effect on EC, K^+, Ca^{2+}, Mg^{2+}, PO_4^{3-} and SO_4^{2} of the soil in both seasons. The 5% to 100% concentrations of the SME significantly ($P<0.01$) affected OC, Fe^{2+}, Cd, Cr, Cu, Mn and Zn contents, while 10% to 100% concentrations of the SME showed significant ($P<0.01$) effect on TKN and Na^+ in the soil in both seasons. Soil pH was significantly ($P<0.01$) affected by the 50% to 100% concentrations of the SME (Tables 5 and 6). 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 were noted to be positively correlated with the SME concentrations in both seasons (Table 7). The contents of Cd, Cr, Cu, Mn and Zn in the soil were increased with the increase in the concentrations of the SME (Table 7). Seasons, SME concentrations and their interaction showed significant ($P<0.01$) effect on Zn, Cd, Cu, Mn and Cr in the soil in both seasons (Table 5). The contamination factor (Cf) indicated the contamination of metals in the soil and it was found in the order of Cr>Cd>Mn>Zn>Cu after the SME irrigation in both seasons (Figure 1).

In the present study, the SME irrigation insignificantly decreased the WHC and BD of the soil in both seasons. The changes in WHC and BD of the soil is likely due to more organic carbon supplied through the sugar mill effluent, which can lower the BD and WHC as also earlier reported by Kumar and Chopra (2010). The SME irrigation significantly increased the EC, OC, TKN, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Zn, Cd, Cr, Cu and Mn in the soil. Maliwal et al. (2004) also reported that the sugar mill effluent irrigation increased the nutrients as well as heavy metals in the soil environment. Chopra et al. (2009) reported that effluent irrigation generally adds PO_4^{3-}, HCO_3^-, Cl^- Na^+, Ca^{2+}, K^+, Mg^{2+}, Zn, Cd, Cr, Cu, Ni and Mn to the soil environment. Kumar and Chopra (2013a) also reported that sugar mill effluent irrigation significantly increased the nutrients and heavy metals in the soil.

The OC in the soil irrigated with the SME was higher than the soil irrigated with bore well water. The more OC in the SME irrigated soil might be due to the higher organic nature of SME. Kumar and Chopra (2012) reported the high OC in the soil irrigated with distillery effluent. The values of TKN, PO_4^{3-} and K^+ in the soil irrigated with the SME were found to be higher than those in the soil irrigated with bore well water and it is likely due to irrigation with TKN, PO_4^{3-} and K^+ rich SME. The contents of Na^+ and SO_4^{2-} were higher in the soil irrigated with SME suggesting a link between soil Na^+ and SO_4^{2-} and higher EC in the SME (Kumar and Chopra, 2013a).

The contents of Cd, Cr, Cu, Mn and Zn in the soil were increased with the increase in SME concentrations. The contents of Cr, Cu, Mn and Zn except Cd were found to be below the maximum levels permitted for Cd (6.0 mg kg^{-1}), Cr (10.0 mg kg^{-1}), Cu (270 mg kg^{-1}) and Zn (600 mg kg^{-1}) for soil in India (BIS, 1991). The contents of heavy

Table 1. Physico-chemical and microbiological characteristics of sugar mill effluent (SME).

Parameter	Effluent concentration (%)							BIS[b] for irrigation water
	0 (BWW)[a]	5	10	25	50	75	100	
TDS (mg L^{-1})	234.00	1386.00*	1810.00**	2268.00**	2524.00**	2892.00***	3060.00***	1900
EC (dS m^{-1})	0.37	2.27**	2.95**	3.83**	3.94**	5.17***	6.96***	-
pH	7.78	7.84ns	7.90ns	7.96ns	8.14ns	8.29ns	8.77ns	5.5-9.0
DO (mg L^{-1})	8.60	6.90s	5.15ns	4.22**	3.90**	1.56***	NIL	-
BOD (mg L^{-1}	4.70	82.64*	144.50**	342.60**	654.70**	960.88***	1264.80***	100
COD (mg L^{-1})	10.32	164.20*	310.58**	755.90**	1472.60**	2150.30***	2874.92***	250
Cl $^{-}$ (mg L^{-1})	68.50	94.62*	178.45**	295.10**	532.80**	780.50***	1070.20***	500
HCO_3^- (mg L^{-1}	290.66	338.70*	476.25*	660.70**	846.87**	1038.45***	1276.44***	-
CO_3^{2-} (mg L^{-1})	116.24	173.90*	348.50*	580.22**	746.90**	960.34***	1155.78***	-
Na^+ (mg L^{-1})	10.30	41.85*	74.84*	148.93**	269.70**	390.40***	548.30***	-
K^+ (mg L^{-1})	7.32	29.45*	56.35*	128.50**	286.90**	420.84***	640.22***	-
Ca^{2+} (mg L^{-1}	28.70	74.60*	124.68*	296.50**	470.34**	645.20***	850.48***	200
Mg^{2+} (mg L^{-1})	16.80	30.48*	62.18*	156.90**	324.75**	487.40***	658.42***	-
TKN (mg L^{-1})	20.86	38.90*	78.95*	180.44**	375.40**	520.98***	740.33***	100
NO_3^{2-} (mg L^{-1})	38.50	65.70*	162.79*	288.96**	490.48**	670.60***	980.77***	100
PO_4^{3-} (mg L^{-1})	0.06	9.60**	24.50**	56.68**	96.50***	140.10***	195.44***	-
SO_4^{2-} (mg L^{-1})	72.45	80.56*	168.40*	340.86**	682.00**	1024.00***	1374.80***	1000
Fe^{2+} (mg L^{-1})	1.80	1.92*	3.86**	8.40***	17.10**	25.87***	34.24***	1.0
Cd (mg L^{-1})	0.04	0.40**	0.82**	2.15***	4.32***	6.55***	8.65***	15
Cr (mg L^{-1})	0.06	0 .62**	1.27**	2.89***	5.68***	8.40***	11.28***	2.00
Cu (mg L^{-1})	0.10	1.30**	2.62**	5.08***	10.32***	15.74***	20.65***	3.00
Mn (mg L^{-1})	0.02	0.97**	1.83**	3.78***	7.46***	11.20***	14.87***	1.00
Zn (mg L^{-1})	0.28	1.75**	3.42**	6.86***	13.64***	20.96***	27.50***	2.00
SPC (SPC mL^{-1})	8.9×10^{2}	7.4×10^{4}**	9.2×10^{5}**	8.7×10^{6}**	6.3×10^{8}***	9.2×10^{10}***	8.4×10^{12}***	10000
MPN (MPN100 mL^{-1})	4.7×10^{2}	6.8×10^{3}**	9.0×10^{3}**	5.9×10^{5}**	8.6×10^{7}***	7.0×10^{9}***	6.2×10^{10}***	5000

ns, *, **,*** non-significant or significant at $P\leq0.05$, or $P\leq0.01$, or $P\leq0.001$, ANOVA.
[a] BWW = bore well water.
[b] BIS = Bureau of Indian standard.
Least Squares Means analysis.

Table 2. ANOVA for effect of SME on soil characteristics.

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

ns, *, ** non-significant or significant at $P\leq0.05$ or $P\leq0.01$, ANOVA.

Table 3. ANOVA for effect of SME on concentrations of cations and anions.

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

*, ** significant at $P\leq0.05$ or $P\leq0.01$, ANOVA.

Table 4. ANOVA for effect of SME on concentrations of metals.

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

*, ** significant at $P\leq0.05$ or $P\leq0.01$, ANOVA.

metals in the SME irrigated soil were higher than the bore well water irrigated soil. Thus, fertigation with SME concentrations considerably increased the nutrients as well as metals in the soil. The findings were supported byBaskaran et al. (2009) who reported the higher contents of Cu (0.75 mgL^{-1}), Fe (21.28 mgL^{-1}), Mn (15.64 mgL^{-1}) and Zn (7.28 mgL^{-1}) in sugar mill effluent irrigated soil.

Effect on germination

At 0-15 days after sowing, the maximum seed

Table 5. Effects of SME concentration and season interaction on physico-chemical characteristics of a loamy soil before and after fertigation of *V. radiata* in both seasons.

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	2.18	7.60	0.52	16.80	144.50	20.42	12.86
	5	2.74ns	8.19ns	1.85*	27.44 ns	162.44 ns	39.54 ns	16.90 ns
	10	2.92ns	8.44ns	3.87*	30.77*	180.33 ns	46.75 ns	24.80 ns
	25	3.04*	8.65ns	4.93**	35.90*	190.40*	80.04*	36.74*
	50	3.19*	8.75*	5.88**	40.10*	120.50*	120.34*	44.80*
	75	3.44*	8.80*	8.06**	43.80**	228.47**	140.96*	68.90*
	100	3.68**	8.86*	9.68**	46.75**	234.80**	160.56**	80.45*
Summer	0	2.19	7.60	0.52	16.94	145.20	20.87	12.95
	5	2.84ns	8.25ns	1.94*	28.05 ns	168.94 ns	42.70 ns	19.55 ns
	10	2.98ns	8.59ns	3.98*	31.97*	186.75 ns	50.38 ns	28.95 ns
	25	3.32*	8.68ns	5.24**	36.82*	197.45*	88.50*	41.95*
	50	3.40*	8.79*	5.97**	41.73*	228.70*	129.45*	50.64*
	75	3.56*	8.85*	8.90**	45.65**	132.30**	148.90*	74.50*
	100	3.77**	8.98*	10.88**	48.95**	240.55**	170.84**	88.45*

ns, *, ** non-significant or significant at $P<0.05$ or $P<0.01$, Least Squares Means analysis.

Table 6. Effects of SME concentration and season interaction on physico-chemical characteristics of a loamy soil before and after fertigation of *V. radiata* 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})	Cd (mg kg^{-1})	Cr (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})
Rainy	0	28.45	46.75	70.40	4.27	0.62	0.80	2.38	0.68	2.68
	5	58.70 ns	71.34 ns	82.40 ns	5.85*	1.52*	2.06*	4.03*	1.57*	5.12*
	10	70.45**	78.94 ns	95.50 ns	6.93*	2.25*	3.87*	4.87*	2.32*	6.20*
	25	130.87**	98.56*	108.59*	8.67**	3.12**	4.99**	6.34**	3.12**	8.70**
	50	187.50**	120.44**	120.84*	9.88**	3.86**	5.94**	7.70**	4.90**	10.66**
	75	250.48**	128.93**	134.66**	11.45**	4.94**	7.23**	9.47**	6.86**	12.90**
	100	298.45**	140.67**	149.75**	13.86**	6.98**	8.77**	10.67**	7.24**	15.56**
Summer	0	28.96	46.90	70.85	4.75	0.62	0.80	2.40	0.70	2.70
	5	62.74 ns	75.50 ns	86.60 ns	5.97*	1.94*	2.24*	4.12*	1.65*	6.60*
	10	76.90**	84.10*	102.76*	7.34*	2.78*	3.87*	5.30*	2.45*	8.43*
	25	138.78**	112.45*	119.50*	9.45**	3.89**	5.10**	7.65**	3.90**	10.72**
	50	194.60**	128.40**	129.47*	10.68**	4.78**	6.55**	8.46**	5.73**	12.55**
	75	268.40**	135.70**	140.38**	12.50**	5.56**	8.32**	10.34**	7.44**	14.56**
	100	310.34**	148.94**	158.94**	14.56**	7.48**	9.45**	12.54**	8.17**	16.80**

ns,*** non-significant or significant at $P<0.01$; Least Squares Means analysis.

germination (96.00%) of *V. radiata* was recorded for control (BWW) and the minimum seed germination (76.00 and 70.00%) of *V. radiata* was observed due to the treatment with 100% concentration of the SME (Figure 2). Germination of *V. radiata* was decreased with the increase in the SME concentrations, and it was recorded to be negatively correlated (r = -0.97) with all the concentrations of the SME in both seasons. The ANOVAindicated that seasons had significant ($P<0.05$) effect on the seed germination and relative toxicity (RT). The maximum RT (126.31% and 137.14%) of the SME against seed germination of *V. radiata* was recorded for the 100% concentration of the SME (Figure 3) and it was noted to be positively correlated (r = +0.60 and r =+0.64) with all the concentrations of the SME in both seasons. The seed germination of *V. radiata* was decreased

Table 7. Coefficient of correlation (r) between SME and soil characteristics in both seasons.

SME/soil characteristics	Season	r - value
SME versus soil WHC	Rainy Summer	-0.97 -0.96
SME versus soil BD	Rainy Summer	-0.95 -0.96
SME versus soil EC	Rainy Summer	+0.98 +0.98
SME versus soil pH	Rainy Summer	+0.92 +0.92
SME versus soil OC	Rainy Summer	+0.97 +0.99
SME versus soil Na^+	Rainy Summer	+0.99 +0.96
SME versus soil K^+	Rainy Summer	+0.97 +0.94
SME versus soil Ca^{2+}	Rainy Summer	+0.95 +0.96
SME versus soil Mg^{2+}	Rainy Summer	+0.97 +0.98
SME versus soil TKN	Rainy Summer	+0.99 +0.99
SME versus soil PO_4^{3-}	Rainy Summer	+0.97 +0.98
SME versus soil SO_4^{2-}	Rainy Summer	+0.96 +0.96
SME versus soil Fe^{2+}	Rainy Summer	+0.98 +0.99
SME versus soil Cd	Rainy Summer	+0.99 +0.99
SME versus soil Cr	Rainy Summer	+0.98 +0.99
SME versus soil Cu	Rainy Summer	+0.98 +0.99
SME versus soil Mn	Rainy Summer	+0.97 +0.98
SME versus soil Zn	Rainy Summer	+0.96 +0.97

when the concentrations of the SME were increased. Although, the maximum seed germination of *V. radiata* was observed with control but the acceptable seed germination (80.00%) of *V. radiata* was recorded from 5% to 25% concentrations of the SME (Figure 2). All the concentrations of the SME and their interaction with seasons affected seed germination of *V. radiata*, and RT of the SME (Table 8). The findings are in accordance with

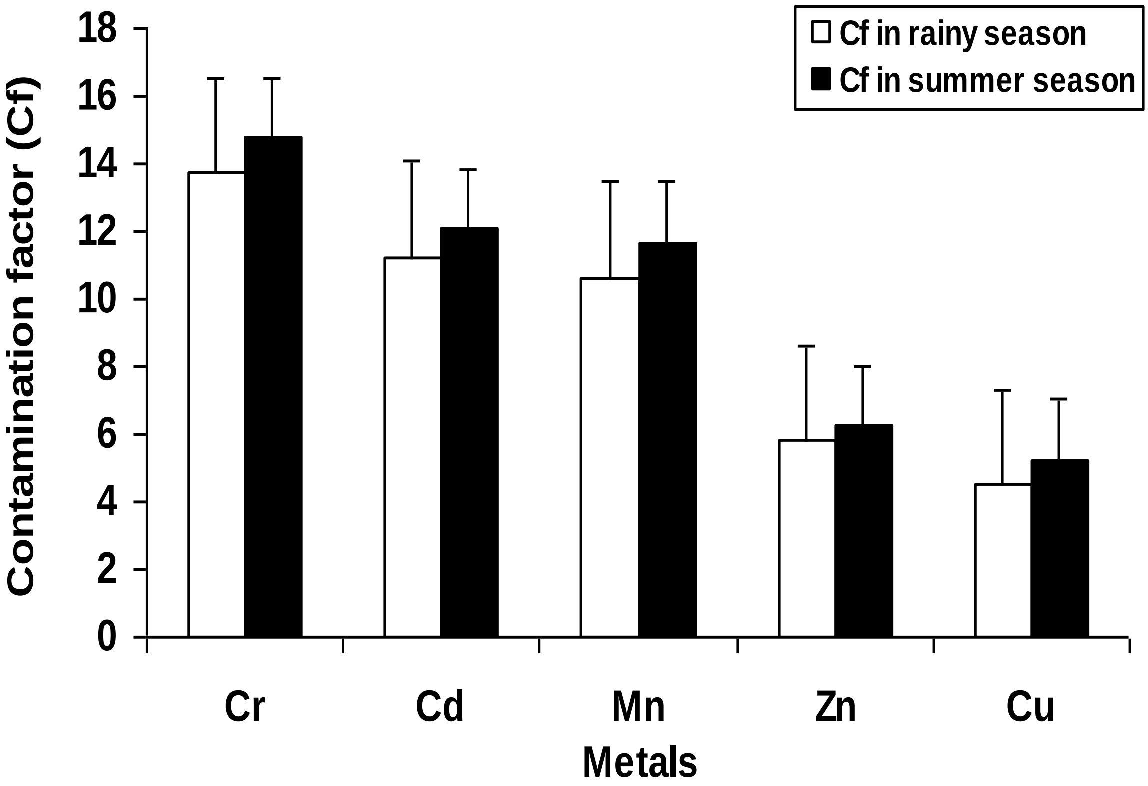

Figure 1. Contamination factor of heavy metals in soil after fertigation with SME. Error bars are standard error of the mean.

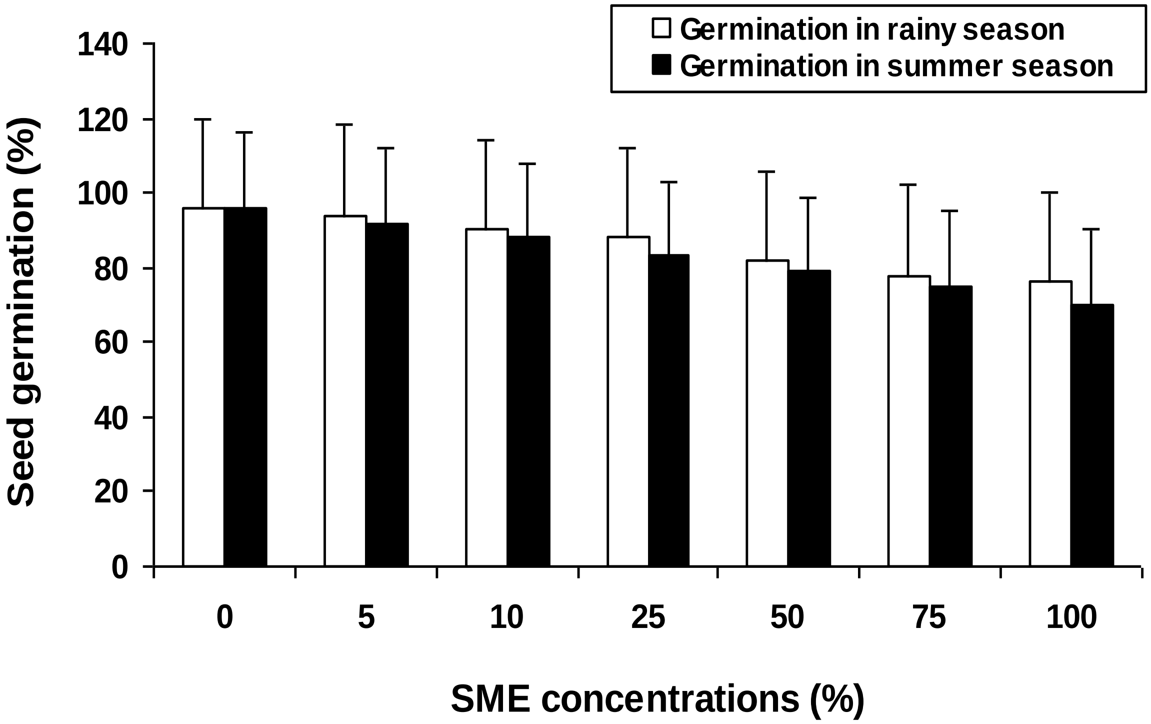

Figure 2. Seed germination of *V. radiata* after irrigation with SME. Error bars are standard error of the mean.

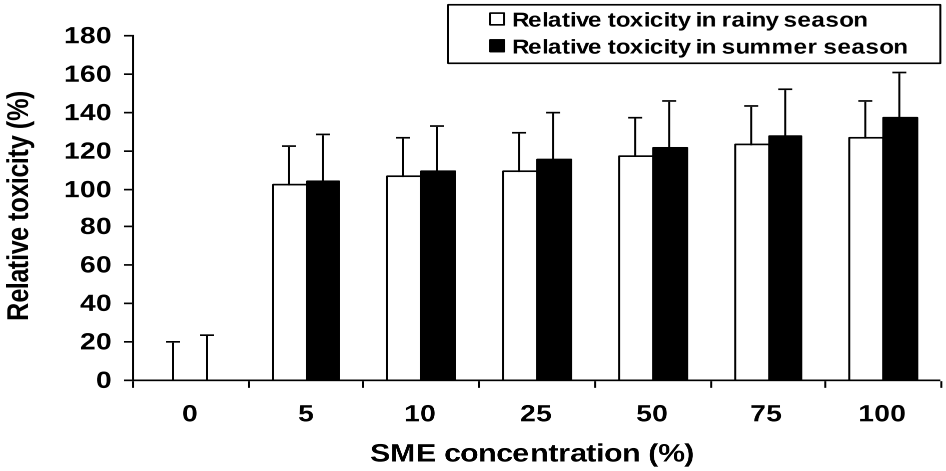

Figure 3. Relative toxicity of SME against seed germination of *V. radiata*. Error bars are standard error of the mean.

Table 8. ANOVA for effect of SME on germination and vegetative growth of *V. radiata*.

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

ns, *, non-significant or significant at P≤0.05, ANOVA, LAI-leaf area index.

Baskaran et al. (2009) who reported that seed germination of green gram (*Vigna radiata* L.) was decreased when the concentrations of the sugar mill effluent increased from 0 to 100%. Lakshmi and Sundaramoorthy (2002) also reported that the seed germination of Ragi (*Eleusine coracana* L.) was decreased when the concentrations of sugar mill effluent increased. Krishna and Leelavathi (2002) reported that the toxicity of sugar mill effluent against the seed germination of paddy (*Oryza sativa* L) was increased with the increase in sugar mill effluent concentrations.

In the present study, the higher concentrations of the SME did not support the seed germination of *V. radiata*. The higher concentrations of the SME lowered the germination of *V. radiata* likely due to the presence of more salts in the effluent at these concentrations. Seed take up water during germination and hydrolyze stored food material and to activate enzymatic systems. NaCl can inhibit the seed germination due to insufficient water absorption (Kumar and Chopra, 2013a, 2014). Seed that absorbs an insufficient amount of water can accumulate 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 sometimes at higher concentrations do not germinate (Chandrasekar et al., 1998). At higher concentrations, more salts can slow the 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 which delays germination (Krishna and Leelavathi, 2002; Kaushik et al., 2004). Thus, 5% to 25% concentrations of the SME favoured the seed germination of *V. radiata*, while 50% to 100% concentrations of the SME inhibited the seed germination of *V. radiata*.

Effect on vegetative growth

The vegetative growth of *V. radiata* at 45 days was affected in both seasons (Table 8). The least shoot length (22.75 and 25.80 cm), root length (10.12 and 13.34 cm), dry weight (6.55 and 7.60 g), chlorophyll content (3.02 and 3.12 mg./g.f.wt) and LAI/plant (2.76 and 2.80) of *V. radiata* were recorded with control, while the moderate shoot length (34.58 and 38.49 cm), root length (12.40 and 15.63 cm), dry weight (7.84 and 8.32 g), chlorophyll content (3.78 and 3.80 mg./g.f.wt) and LAI/plant (3.45 and 3.58) of *V. radiata* were observed with 100% concentration of the SME in both seasons.

The most shoot length (40.12 and 43.20 cm), root length (16.22 and 18.75 cm), dry weight (9.54 and 10.02 g), chlorophyll content (4.87 and 5.30 mg./g.f.wt) and

Table 9. ANOVA for effect of SME on flowering and maturity stage of *V. radiata*.

Source	No. of flowers/plant	No. of pods	Crop yield/plant	HI
Season (S)	ns	ns	ns	ns
SME concentration (C)	ns	ns	ns	ns
Interaction S × C	ns	ns	ns	ns

ns, non-significant, HI-harvest index.

LAI/plant (5.10 and 5.60) of *V. radiata* were due to the treatment with the 25% concentration of the SME in both seasons. The ANOVA indicated that the SME concentrations significantly ($P<0.05$) affected the shoot length, chlorophyll content and LAI/plant of *V. radiata* (Table 8). The seasons had no significant ($P>0.05$) effect on the shoot length, root length, dry weight, chlorophyll content and LAI of *V. radiata*. The interaction of the seasons and SME concentrations only significantly ($P<0.05$) affected the shoot length, chlorophyll content and LAI of *V. radiata* (Table 8). The shoot length, dry weight, chlorophyll content and LAI/plant of *V. radiata* were found to be positively correlated with all the concentrations of the SME in both seasons (Table 11). The root length was noted to be positively correlated with the SME concentrations in the rainy season while it was found to be negatively correlated in the summer season (Table 11).

At the vegetative growth stage, the maximum agronomic growth namely, shoot length, dry weight, chlorophyll content and LAI/plant of *V. radiata* was noted with 25% concentration of the SME. The vegetative growth of *V. radiata* was increased at lower concentrations, that is, from 5% to 25% concentrations of the SME and lowered at higher concentrations, that is, 50% to 100% concentrations of the SME. A higher EC indicates higher salts in the higher concentrations (50% to 100%) of the SME, which lowered the shoot length, root length, dry weight, chlorophyll content and LAI/plant of *V. radiata*. Thus, at lower concentrations (that is, 5% to 25%) of the SME, the nutrients and heavy metals may provide better and more effective stimulation to the agronomic performance of *V. radiata*, while at higher concentrations (that is, 50% and 100%) of the SME, inhibiting the overall performance of the crop plants. The vegetative growth is associated with the development of new shoots, twigs, leaves and leaf area. The shoot length, root length, dry weight and LAI/plant of *V. radiata* were observed higher at 25% concentration of the SME, and it may be due to optimal 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 the cell enlargement and in triggering young tissues or mersitematic growth (Swamy et al., 2001). The chlorophyll content was noted higher due to use of 25% concentration of the SME in both seasons, and is likely due to Fe, Mg and Mn contents in the SME, which are associated with chlorophyll synthesis (Porra, 2002). The 25% concentration of the SME contains optimum contents of nutrients required for maximum vegetative growth of *V. radiata*. Kumar and Chopra (2010) reported that the growth, chlorophyll and protein contents in French bean (*Phaseolus vulgaris* L.) were increased at lower concentrations, that is, from 5% to 50% concentrations of the SME and decreased at higher concentrations, that is, from 75% and 100% concentrations of the SME. The findings were also supported by Kaushik et al. (2004) who reported that the growth of wheat plants (*Triticum aestivum* L.) was decreased when the concentration of sugar mill effluent increased.

Effect on flowering

The ANOVA indicated that seasons, SME concentrations and interaction of the seasons and SME concentrations had insignificant ($P>0.05$) effect on the number of flowers/plant of *V. radiata* (Table 9). At the flowering stage (60 days after sowing) the most flowers (48.00 and 56.00) of *V. radiata* was recorded with 25% concentration of the SME in both seasons. Numbers of flowers were decreased when concentrations of the SME increased. The number of flowers was found to be positively correlated with all the concentrations of the SME (Table 11). The least numbers of flowers/plant 32.00 and 38.00 were noted with control and moderate number of flowers 42.00 to 45.00 of *V. radiata* was observed with 100% concentration of the SME in both seasons.

The number of flowers/plant was increased from 5% to 25% and decreased from 50% to 100% concentrations of the SME in both seasons. Thus, 25% concentration of the SME favoured the flowering of *V. radiata*. The findings were in accordance with Kumar and Chopra (2010) who reported that the number of flowers of French bean (*Phaseolus vulgaris* L.) was increased at lower concentrations, that is, from 5% to 25% and decreased at higher concentration, that is, from 50% to 100% of distillery effluent. 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%

Table 10. ANOVA for effect of SME on concentrations of metals in *V. radiata*.

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

ns, *, ** non-significant or significant at P≤0.05 or P≤0.01, ANOVA.

concentration of SME; it might be due to the fact that this concentration contains sufficient nitrogen and phosphorus. Furthermore, P and K prevent flower abortion so pod formation occurs (El-Naggar, 2005). The numbers of flowers of *V. radiata* were decreased at higher concentrations of the SME. This is likely due to more contents of heavy metals in the soil, which inhibits uptake of P and K by plants at higher concentrations of the SME (Pandey et al., 2008).

Effect on maturity

At the maturity stage (90 days after sowing), the numbers of pods/plant, crop yield/plant and harvest index (HI) were not significantly (P>0.05) affected by seasons, SME concentrations and their interaction (Table 9). The least number of pods/plant (29.00 and 36.00), yield/plant (28.20 and 32.50 g) and HI (427.63 and 430.53%) were observed with the control while the moderate number of pods/plants (37.00 and 40.00); yield/plant (35.60 and 38.90 g) and HI (454.08 and 467.54%) were noted with 100% concentration of the SME in both seasons. The most number of pods/plants (45.00 and 52.00), yield/plant (45.58 and 48.80g) and HI (477.77 and 487.02%) were recorded with 25% concentration of the SME in both seasons. The numbers of pods/plant, crop yield/plant and harvest index (HI) were noted to be positively correlated with all the concentrations of the SME (Table 11).

At the maturity stage, the most numbers of pods/plant, crop yield/plant and HI of *V. radiata* were noted with 25% concentration of the SME in both seasons. The role of K, Fe, Mg and Mn at maturity is important and associated with synthesis of chlorophyll, and enhances the formation of pods at harvest (El-Naggar, 2005; Porra, 2002). 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% concentration of the SME favored the pods formation and crop yield of *V. radiata.* This is likely due to the presence of K, Fe, Mg and Mn contents in 25% concentration of the SME; while higher concentrations of the SME lowered the pod formation and crop yield of *V. radiata*. Therefore, at the maturity stage the agronomic performance of *V. radiata* was gradually increased at lower concentration, that is, from 5% to 25% concentrations of the SME and decreased at higher concentrations, that is, 50% to 100% concentrations of the SME.

Effect on biochemical constituents and heavy metals

The ANOVA indicated that seasons, SME concentrations and their interaction significantly (P>0.05) affected all the metals Cd, Cr, Cu, Mn and Zn and biochemical constituents like crude fiber, and total carbohydrates in *V. radiata* (Table 10). The 25% to 100% concentrations of the SME affected Cd, Cr, Cu, Mn and Zn contents in *V. radiata.* More irrigation could lead to increase of metals in tissues. The Cd, Cr, Cu, Mn and Zn contents in *V. radiata* were highest with 100% concentration of the SME (Figures 4 and 5). The Cf of various metals was in the order of Mn>Zn>Cu>Cr>Cd in *V. radiata* after irrigation with the SME (Figure 6). The most Cf was noted for Mn; the least was for Cd in *V. radiata* with 100% concentration of the SME in both seasons. The contents of these metals in *V. radiata* were observed to be positively correlated with all the concentrations of the SME in both seasons (Table 11). The most crude proteins, crude fiber and total carbohydrates were recorded with 25% concentration of the SME in both seasons (Figures 7 to 9). Content of crude proteins (r = +21 and r = +0.22), crude fiber (r = +0.18 and r = +0.22) and total carbohydrates (r = +0.41 and r = +0.48) were noted to be positively correlated with all of the concentrations of the SME in both seasons. The Cf was significantly (P<0.05) affected in both seasons.

The most contents of Cd, Cr, Cu, Mn and Zn in *V. radiata* were found with 100% concentration of the SME. The accumulation of Cu, Mn and Zn except Cd and Cr in *V. radiata* was noted below the permissible limit of FAO/WHO standards for Cd (0.20 mg Kg $^{-1}$), Cr (2.30 mg Kg $^{-1}$), Cu (40.00 mg Kg $^{-1}$) and Zn (60.00 mg Kg $^{-1}$) (FAO/WHO, 2011). The heavy metals were found more at the higher concentrations (that is, 50% to 100%) of the SME and likely inhibited growth of *V. radiata*.

The 25% concentration of the SME favored the vegetative growth, flowering and maturity of *V. radiata*. This is likely due to optimal uptake of these metals by crop plants, which supports various biochemical and physiological processes. Baskaran et al. (2009) also reported that the higher concentrations of sugar mill effluent inhibited the growth of green gram (*Vigna radiata* L.) due to the presence of more heavy metals in the sugar mill effluent. The contents of crude proteins, crude fiber and total carbohydrates in *V. radiata* were observed with 25% concentration of the SME and these were decreased from 50% to 100% concentrations of the SME.

Table 11. Coefficient of correlation (r) between SME and *V. radiata* in both seasons.

SME / *V. radiata*	**Season**	**r - value**
SME versus shoot length	Rainy	+0.70
	Summer	+0.72
SME versus root length	Rainy	+0.21
	Summer	+0.19
SME versus dry weight	Rainy	+0.42
	Summer	+0.44
SME versus chlorophyll content	Rainy	+0.52
	Summer	+0.56
SME versus leaf area index (LAI)	Rainy	+0.58
	Summer	+0.62
SME versus no. of flowers/plant	Rainy	+0.50
	Summer	+0.53
SME versus no. of pods	Rainy	+0.56
	Summer	+0.58
SME versus crop yield/plant	Rainy	+0.24
	Summer	+0.26
SME versus harvest index (HI)	Rainy	+0.49
	Summer	+0.51
SME versus Cd	Rainy	+0.97
	Summer	+0.98
SME versus Cr	Rainy	+0.96
	Summer	+0.97
SME versus Cu	Rainy	+0.98
	Summer	+0.99
SME versus Mn	Rainy	+0.99
	Summer	+0.99
SME versus Zn	Rainy	+0.98
	Summer	+0.99

Vijayaragavan et al. (2011) reported the maximum total sugar, amino acids and protein contents in radish (*Raphanus sativus* L.) with 20% concentration and the content of amino acids and protein was decreased from 40% to 100% concentrations of sugar mill effluent.

Conclusions

The present investigation concluded that the SME irrigation increased the nutrients and heavy metals of the soil in both seasons. The SME irrigation significantly changed the soil quality and affected the natural composition of the soil. Such alterations improved the fertility and enhanced the nutrient status of the soil at lower concentrations of SME irrigation. The contents of Cu, Mn and Zn except Cd and Cr in the soil and *V. radiata* were noted under the permissible limit of Indian soil standards and FAO/WHO standards respectively.

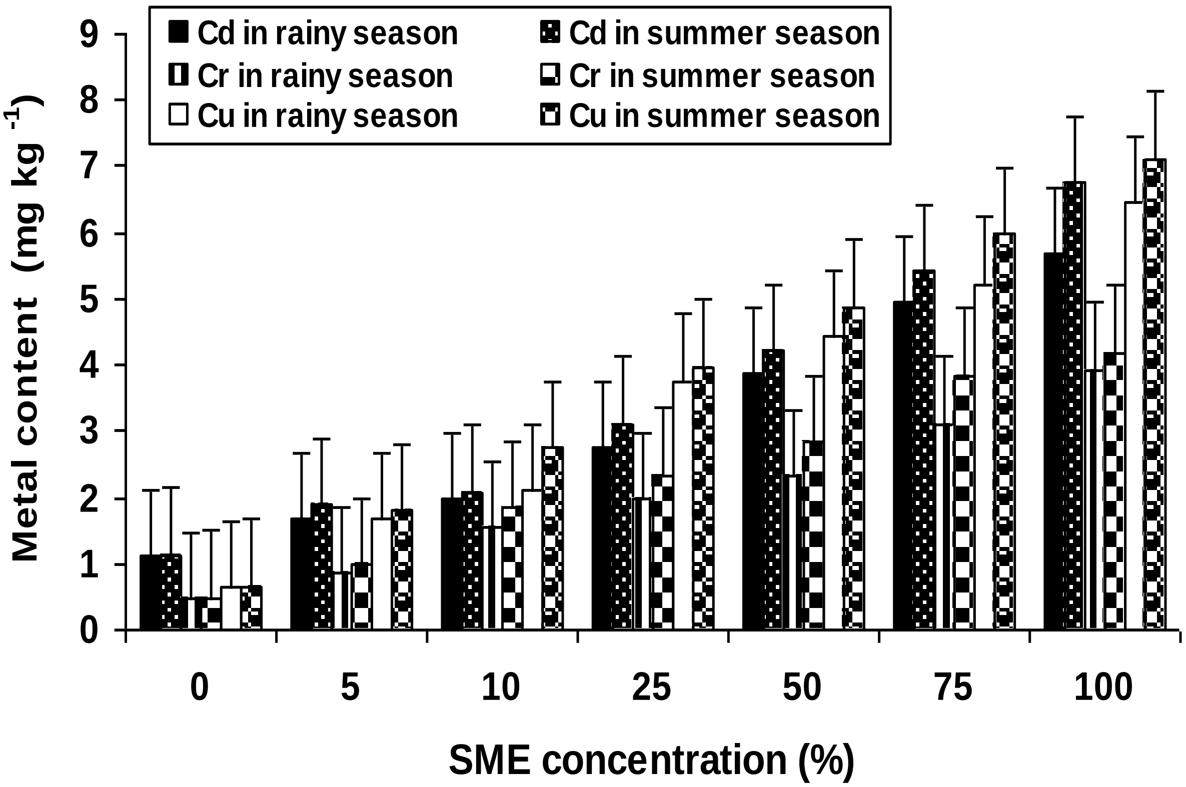

Figure 4. Content of Cd, Cr and Cu in *V. radiata* after fertigation with SME.
Error bars are standard error of the mean.

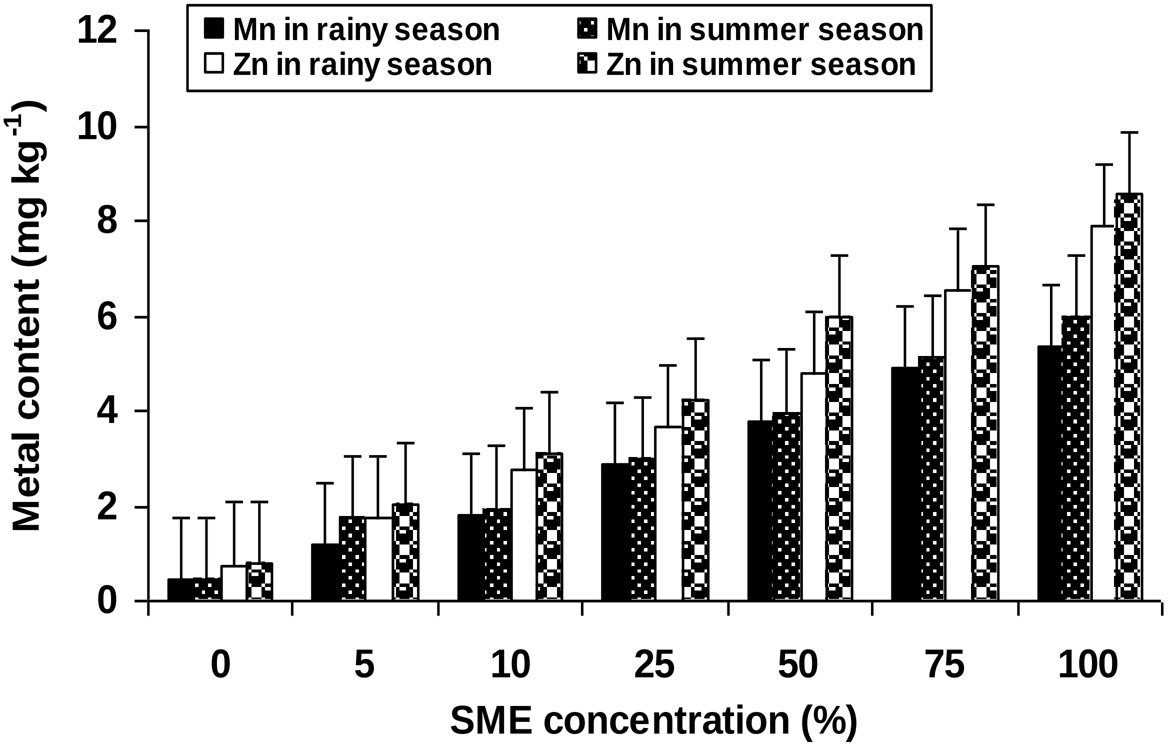

Figure 5. Content of Mn and Zn in *V. radiata* after fertigation with SME.
Error bars are standard error of the mean.

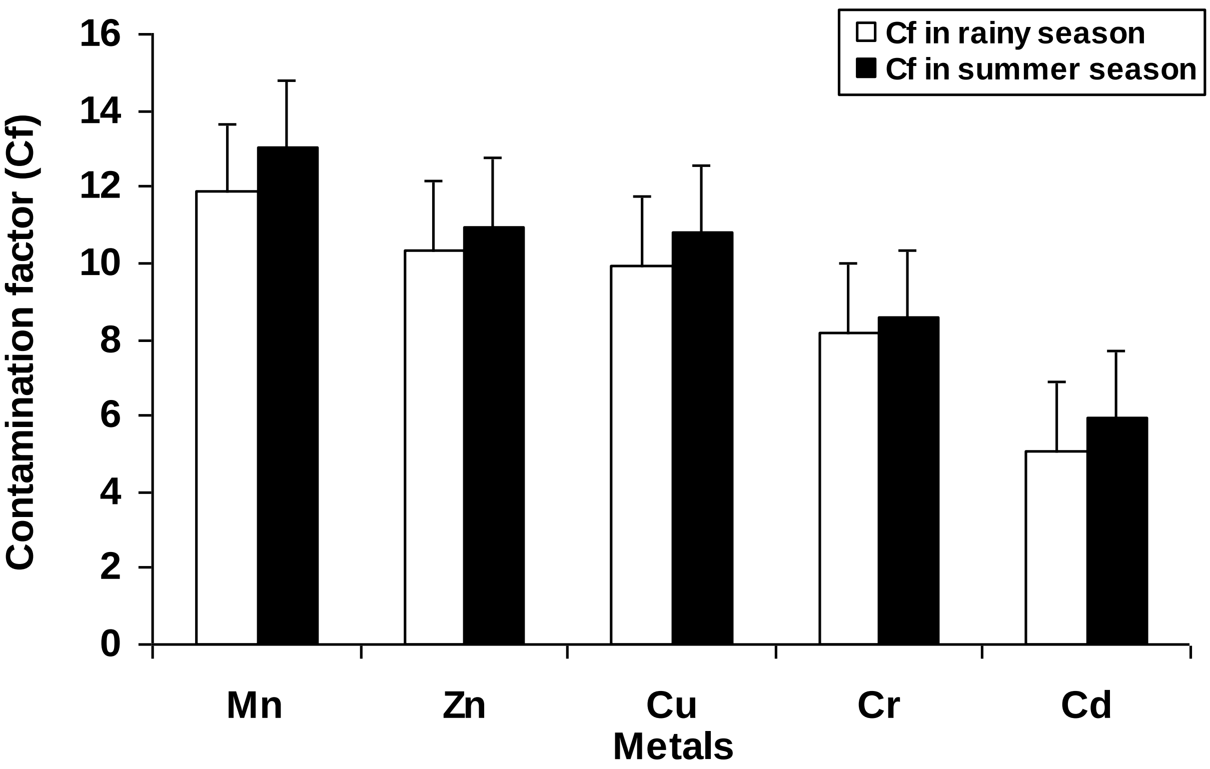

Figure 6. Contamination factor of heavy metals in *V. radiata* after fertigation with SME. Error bars are standard error of the mean.

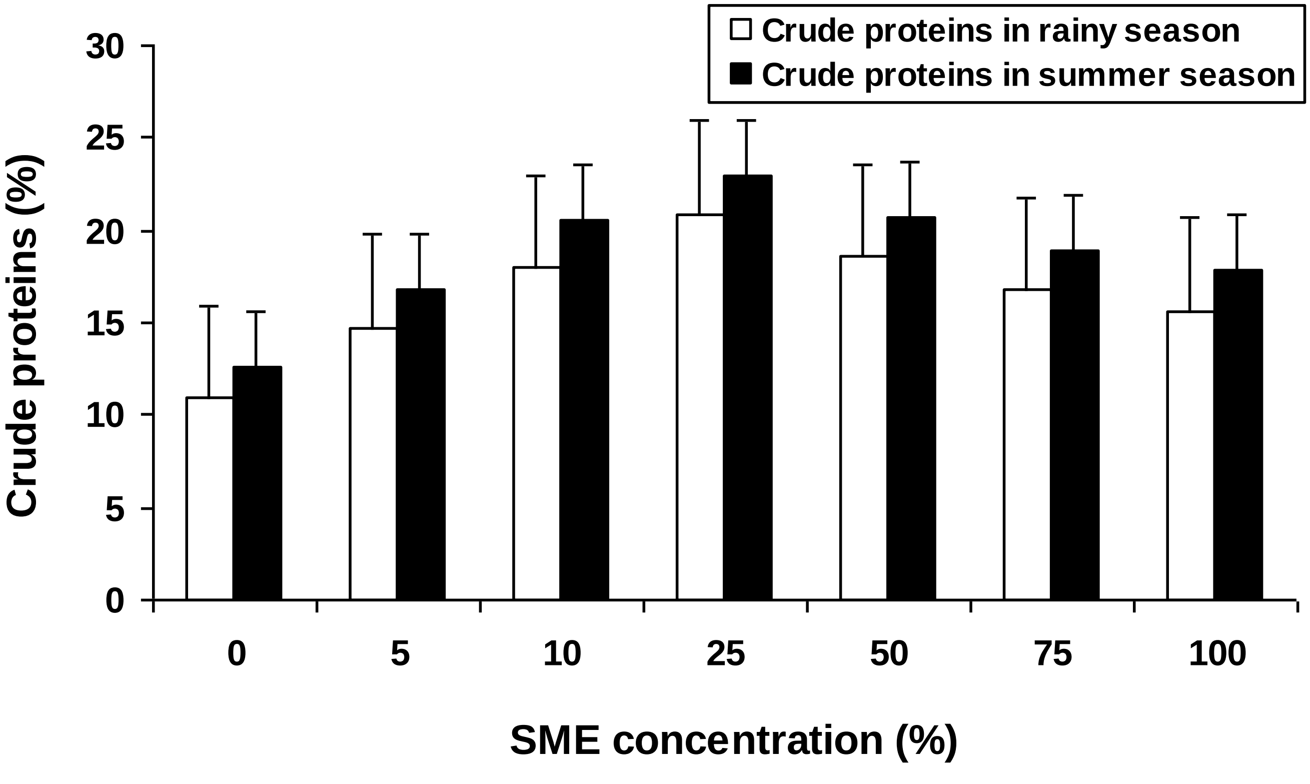

Figure 7. Crude proteins in *V. radiata* after fertigation with SME. Error bars are standard error of the mean.

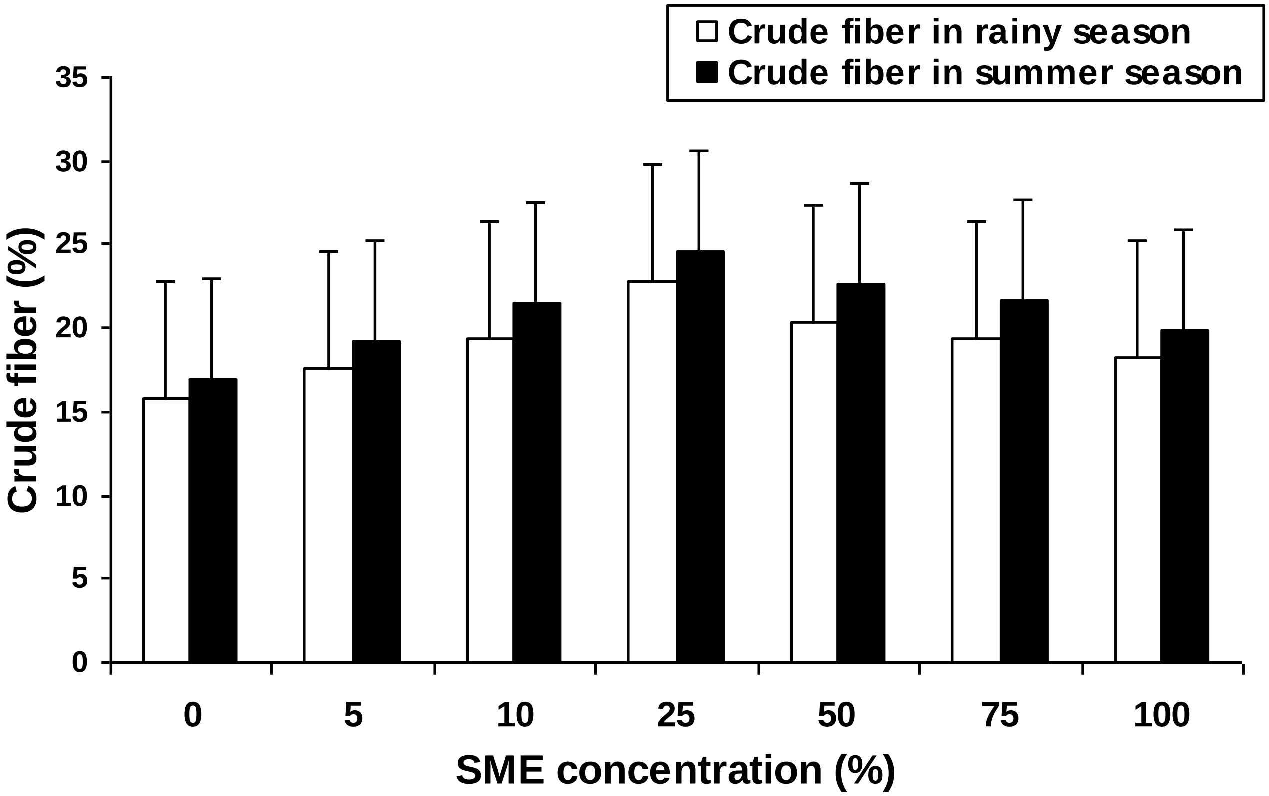

Figure 8. Crude fiber in *V. radiata* after fertigation with SME.
Error bars are standard error of the mean.

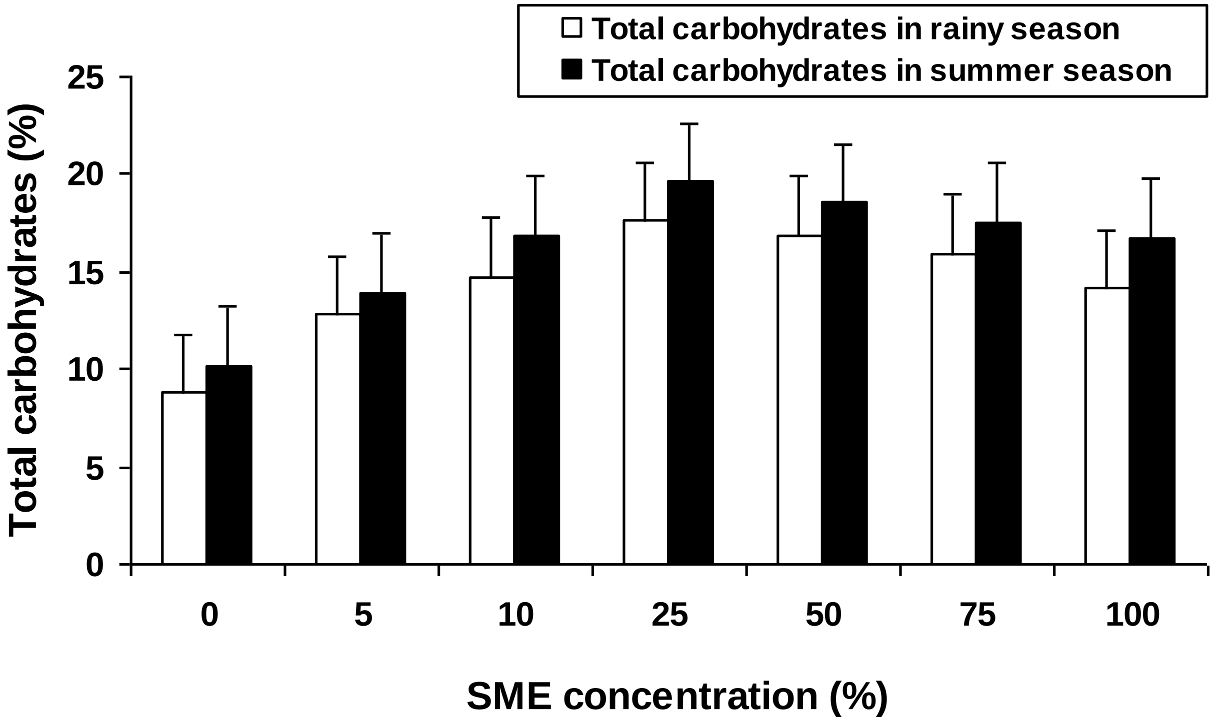

Figure 9. Total carbohydrates in *V. radiata* after fertigation with SME.
Error bars are standard error of the mean.

The optimum agronomic growth of *V. radiata* was recorded with 25% concentration of the SME. Therefore, dilution of the SME is necessary to minimize the toxicity of various heavy metals present in the SME. It appears that the SME have the potentiality as organic fertilizer and can be used as an agro-based biofertigant after appropriate dilution to improve the crop yield. Further studies on the agronomic growth and changes in biochemical composition of *V. radiata* after the SME irrigation are required.

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