International Journal of Agricultural Policy and Research Vol.1 (5), pp. 131-141, July 2013
Available online at http://www.journalissues.org/journals-home.php?id=1

Original Research Paper

Enrichment and translocation of heavy metals in soil and plant of *Vicia faba* L. (Faba bean) after fertigation with distillery effluent

Accepted 24 June, 2013

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This investigation studies the enrichment and translocation of heavy metals in soil and plan of V. *faba* (Faba bean, var. Pusa Sumit) after fertigation with distillery effluent. A field experiment was conducted in the experimental garden of Gurukula Kangri University, Haridwar, during the year 2011 and 2012. Doses of distillery effluent viz. 10%, 25%, 50%, 75% and 100% were used for fertigation of *V. faba* along with bored deep tubewell water (control). The results revealed that distillery effluent had significant ($P<0.01$) effect on soil characteristics viz. EC, pH, Cl^-, 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. The soil characteristics were recorded to positively correlate with different concentration of distillery effluent. The agronomical characteristics viz. shoot length, root length, number of flowers, pods, dry weight, chlorophyll content, leaf area index (LAI), crop yield and HI of *Vica faba,* increased with irrigation of distillery effluents from 10% to 25% and decreased with the increase in effluent concentration from 50% to100% as compared to control. Maximum performance of agronomic characteristics of *V. faba* was recorded with 25% concentration of distillery effluent. The biochemical components viz. crude proteins, crude fiber and crude carbohydrates were noted maximum with 25% concentration of distillery effluent. The enrichment factor of heavy metals were in the order of Zn>Mn>Cr>Cd>Cu for soil and Cu>Zn>Mn>Cr>Cd in plant of *V. faba* after fertigation with distillery effluent. The translocation of various heavy metals in different plant parts of *V. faba* were in order of leaves>shoot>root> fruits for Cu, Mn and Zn, root>shoot>leaves>fruit for Cd and shoot>root>leaves>fruit for Cr after distillery effluent irrigation. Thus the distillery effluent with appropriate dilution can be used as a bio-fertigant for V. *faba*.

Key words: Distillery effluent, faba bean, fertigation, agronomical practices, heavy metals, enrichment, translocation

ABBREVIATIONS

ANOVA-Analysis of variance;
BIS- Bureau of Indian standards;
RT- Relative toxicity;
LAI- Leaf area index;
HI- Harvest index

INTRODUCTION

In the past two decades, there has been a notable increase in the use of wastewater for crop irrigation, especially in arid and seasonally arid areas of both industrialized and developing countries (Baghel, 2008; Biswas et al., 2009; Chidankumar et al., 2009). It may be due to the increasing scarcity of alternative water for irrigation and high cost of chemical fertilizers (Ramana et al., 2002; Sukanya and Meli, 2004; Rath et al., 2011). In agriculture, irrigation water quality is believed to have effects on the soils and crops (Bharagava et al., 2008; Biswas et al., 2009; Tharakeshwari and Jagannath, 2011a; Mohamed and Ebead, 2013).
The use of saline water may result in the reduction of crop

yield, while the sodic water may deteriorate the physical properties of the soil with consequent reduction in the yield (Chandra et al., 2009; Chidankumar et al., 2009; Shenbagavalli et al., 2011). Thus considerable attention is presently focused towards the aspects of wastewater application, including the possible causes of potentially harmful agents in the environment (Chonker et al., 2000; Hati et al., 2007; Kalaiselvi et al., 2009). Although, water effects on soils, crops and water management are of more concern to people when the irrigant is wastewater, which may contain agents capable of inducing adverse effects on the soil media and the agricultural products (Kaushik et al., 2005; Nath et al., 2007; Kannan and Upreti, 2008).

The disposal of wastewater is a major problem faced by industries, due to its generation of high volume of effluent and with limited space for land based treatment and disposal (Pandey et al., 2008; Chidankumar et al., 2009; Shenbagavalli et al., 2011). On the other hand, wastewater is also a resource that can be applied for productive uses, since wastewater contains nutrients that can be used for the cultivation of agricultural crops (Hati et al., 2007; Chandra et al., 2009; Rath et al., 2011). Irrigation with effluents is known to contribute significantly to the heavy metals content of soil as well as crop plants (Kumar and Chopra, 2012). 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 (Hati et al., 2007; Bharagava et al., 2008; Chopra et al., 2009). Vegetables take in 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 that consume them (Muchuweti et al., 2006; Bharagava et al., 2008). India has nearly 330 distilleries, producing about 3,500 million liter alcohol (Kaul et al., 1995; Hati et al., 2007). They generate a huge amount of wastewater (spent wash) with high chemical oxygen demand (COD) and biochemical oxygen demand (BOD).

Faba bean (*Vicia faba*) is one of the most important winter vegetable and pulse crops for human consumption in the Middle East (Gutierrez et al., 2006; Singh et al., 2012; Mohamed and Ebead, 2013). It is used green or dried, fresh or canned, and for stock feed. The roasted seeds of Faba bean are eaten like peanuts in India. Feed value of Faba beans is high, as it is rich in protein, vitamins, minerals and fibers (Grashoff, 1990; Martin et al., 1991; Mohamed and Ebead, 2013). Faba beans have also been considered as a meat extender or substitute and as a skim-milk substitute. It also grown for green manure, but more generally for stock feed. It also has medicinal values used for diuretic, expectorant, and tonic (Sprent et al., 1977; Martin et al., 1991).

The utilization of industrial waste as soil amendment and irrigation of agricultural crops has generated interest in recent times (Kumar, 2010). Studies have been conducted on the characteristics of industrial effluents and their effects on soil properties and agricultural crops (Ramana et al., 2002; Kaushik et al., 2005; Muchuweti et al., 2006; Hati et al., 2007; Bharagava et al., 2008; Kannan and Upreti, 2008; Kumar, 2010; Mohamed and Ebead, 2013). But much attention has not been paid so far on the use of distillery effluent on agronomical practices of faba bean (*V. faba*). Keeping in view the problem of wastewater disposal, scarcity of irrigation water and re-use of wastewater effluent, their effect on soil quality, accumulation of heavy metals and economic importance of *V. faba*, the present investigation is undertaken to study the enrichment and translocation of heavy metals in soil and *Vicia faba* L. (Faba bean) after fertigation with distillery effluent.

MATERIALS AND METHODS

Experimental design

A field study were conducted in the Experimental Garden of the Department of Zoology and Environmental Sciences, Faculty of Life Sciences, Gurukula Kangri University Haridwar, India (29º55'10.81'' N and 78º96 07'08.12'' E), during the year 2011 and 2012. Six plots (each plot had an area of 9 m^2) were selected for six treatments of distillery effluent viz. 0% (control), 10, 25, 50 75 and 100% for the cultivation of *V. faba.* The experiment were conducted under completely randomized design and replicated three times.

Effluent collection and analysis

Shamli Distillery and Chemical Works, Shamli (Uttar Pradesh) were selected for the collection of effluent samples. The treated effluents were collected from outlet of the effluent treatment plant situated in the campus of the distillery. The effluent were brought to the laboratory and analyzed for various physico-chemical and microbiological parameters viz. TDS, pH, electrical conductivity (EC), dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), chlorides (Cl^-), bicarbonates (HCO_3^-), carbonates (CO_3^{-2}), sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), total Kjeldahl nitrogen (TKN), nitrate (NO_3^{2-}), phosphate (PO_4^{3-}), iron (Fe), cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), zinc (Zn), standard plate count (SPC) and most probable number (MPN) following standard methods (APHA, 2005) and further used for fertigation of *V. faba*.

Sowing of seeds, irrigation pattern and soil analysis

The seeds of *Vica faba* (var. Pusa Sumit) were procured from ICAR, Pusa, New Delhi and sterilized with 0.01% mercuric chloride and soaked for 12 hrs. Seeds were sown in 10 rows with a distance of 30.0 cm between rows, while distances between the seeds were 15 cm. The thinning was done manually after 15 days of germination to maintain the desired plant spacing and to avoid competition between plants. Plants in each plot was fertigated twice in a month with 50 gallons of distillery effluent with 10, 25, 50, 75 and 100% treatments, along with bore well water as the control. The soil was analyzed before sowing and after harvesting of crop for various physico-chemical parameters viz. bulk density (BD), water holding capacity (WHC), soil texture, EC, pH , Cl^-, OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, TKN, PO_4^{3-} and SO_4^{2-}, Cd, Cr, Cu, Mn and Zn following standard methods cited by (Chaturvedi and Sankar (2006)).

Study of crop parameters

Agronomical parameters of *Vica faba* from germination to maturity (0-90 days) were determined following standard methods (Chandrasekar et al., 1998 for seed germination, relative toxicity, shoot length, root length, number of flowers number of pods and crop yield); (Milner and Hughes, 1968 for dry weight), (Porra, 2002 for chlorophyll content), (Denison and Russotti, 1997 for leaf area index, LAI) and (Sinclair, 1998 for Harvest index, HI). The nutrients quality of *V. faba* were determined using the following parameters; crude protein (4.204 AOAC, 1980), crude fiber (4.601 AOAC, 1980) and the total carbohydrate in dry matter determined by the anthrone reagent method (Cerning and Guilhot, 1973)

Extraction of heavy metals and their analysis

For heavy metal extraction 10 ml sample of distillery effluent, and 1.0 g of air dried soil or plants were taken in digestion tubes with 3 ml of conc. HNO_3 and digested in an electrically heated block for 1 hour at 145° C. To this mix 4 ml of $HClO_4$ was added and heated to 240°C for 1 hour. The mixture was cooled and filtered through Whatman # 42 filter paper and aliquot was made to 50 ml with double distilled water and used for analysis. Heavy metals were analyzed using an Atomic Absorption Spectrophotometer (AAS) following methods of Chaturvedi and Sankar (2006). The enrichment factor for heavy metals accumulated in distillery effluent irrigated soil and *V. faba* was calculated following Kim and Kim (1999).

Statistical analysis

Data were subjected to two-way ANOVA by using SPSS (ver. 12.0, SPSS Inc., Chicago, Ill.).

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 were plotted with Sigma plot (ver. 12.3, Systat Software, Inc., Chicago, IL).

RESULTS AND DISCUSSION

Characteristics of distillery effluent

The characteristics of distillery effluent at various concentrations are presented in Table 1. The maximum values of various parameters were recorded with 100% concentration of distillery effluent. The effluent was reddish in colour with odour of molasses. The higher values of TDS showed positive correlation with EC of the effluent. Chlorides, carbonates and bicarbonates were higher in distillery effluent. It was acidic in nature. The low pH of distillery effluent might be due to the presence of higher concentration of organic acids such as CH_3COOH (Kumar, 2010; Rath et al., 2011). The effluent were rich in organic and inorganic nutrients like Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, TKN, NO_3^{2-}, PO_4^{3-} and SO_4^{2-} and were far- beyond the prescribed limit of Indian Irrigation Standards (BIS, 1991). High BOD, COD, TKN, NO_3^{2-} and PO_4^{3-} might be due to presence of high oxidizable organic matter, and rapid consumption of dissolved inorganic materials (Biswas et al., 2009; Kumar and Chopra, 2012). The content of HCO_3^-, CO_3^{2-}, Na^+, K^+, Mg^{2+} and SO_4^{2-} were also found slightly higher in effluent, which is associated with higher EC of the distillery effluent (Bharagava et al., 2008). The content of heavy metals viz. Cd, Cr, Cu, Mn and Zn were also found to be higher in distillery effluent than the permissible limit given by Bureau of Indian Standard (Table 1). Total bacterial count and most probable number showed higher load of microbial species in distillery effluent and it's likely due to higher organic load of distillery effluent (Kumar and Chopra, 2012).

Effect of effluent irrigation on soil characteristics

The soil characteristics after irrigation of *V. faba* with different concentrations of distillery effluent are presented in Table 2. The soil texture (loam; 40% sand: 40% silt: 20% clay) were observed unchanged with the application of all concentrations of distillery effluent throughout the period of study. Significant increase in the 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 might be attributed to higher organic load of distillery effluent. Similarly, earlier studies of soil irrigated with distillery effluent also showed an increase for these parameters as reported by Kumar and Chopra (2012). The findings were in accordance with Rath et al. (2011).

In addition, the higher values of EC of distillery effluent irrigated soil indicated enrichment of soluble cations and anions such as Na^+, K^+, Ca^+, Mg^{2+}, Cl^-, PO_4^{3-} and SO_4^{2-} through the use of effluent in different concentrations (Kumar and Chopra, 2012). In the present study, there was a maximum increase in the EC (5.86 dS m^{-1}) of soil as the concentration of DE increased up to 100%. This might be due to increase in concentration of potassium salts, which are mainly responsible for increasing the EC of the distillery effluent

Table1. Physico-chemical and microbiological characteristics of distillery effluent.

Parameter	Bore well water (Control)	Effluent concentration (%)					BIS[a] for irrigation water
		10	25	50	75	100	
TDS(mg L^{-1})	236.80	874.00	2040.80	2564.00	4468.00	7798.48	1900
EC(dS m^{-1})	0.38	1.46	3.45	3.86	7.36	13.48	-
pH	7.66	7.60	7.52	6.88	6.56	5.96	5.5-9.0
DO(mg L^{-1})	8.64	4.76	4.92	3.12	1.24	NIL	-
BOD(mg L^{-1})	3.96	338.70	864.58	1644.74	2488.96	3276.84	100
COD(mg L^{-1})	5.92	906.36	2248.74	4385.56	6522.40	8678.65	250
Cl^-(mg L^{-1})	18.94	194.50	474.78	864.96	1260.40	1680.66	500
HCO_3^- (mg L^{-1})	184.60	196.30	228.75	326.40	425.74	536.96	-b
CO_3^{-2} (mg L^{-1})	60.86	86.44	94.36	136.50	178.96	236.80	-
Na^+ (mg L^{-1})	9.60	28.64	72.42	142.30	229.69	287.94	-
K^+ (mg L^{-1})	5.62	68.75	166.48	286.96	425.64	548.68	-
Ca^{2+} (mg L^{-1})	24.75	224.88	496.69	962.75	1434.80	1876.86	200
Mg^{2+}(mg L^{-1})	12.86	48.64	98.48	164.36	236.86	298.70	-
TKN (mg L^{-1})	22.30	78.64	142.69	326.80	464.36	580.97	100
NO_3^{2-} (mg L^{-1})	28.66	178.68	436.72	776.56	1144.86	1468.78	100
PO_4^{3-} (mg L^{-1})	0.04	68.76	172.36	329.82	482.30	642.26	-
SO_4^{2-} (mg L^{-1})	16.36	162.54	312.41	642.36	966.80	1286.49	1000
Fe^{2+} (mg L^{-1})	0.32	3.26	7.88	16.44	24.36	32.12	1.0
Cd (mg L^{-1})	BDL[c]	0.74	3.87	7.20	10.36	14.96	15
Cr (mg L^{-1})	BDL	0.29	0.74	1.84	3.24	7.86	2.00
Cu (mg L^{-1})	0.04	0.56	1.13	2.43	5.96	11.36	3.00
Mn (mg L^{-1})	0.02	0.21	0.45	0.93	1.92	3.94	1.00
Zn (mg L^{-1})	0.04	0.56	1.14	2.30	4.62	9.22	2.00
SPC(SPC ml^{-1})	3.64×10^2	5.36×10^5	8.55×10^6	7.23×10^7	5.88×10^8	8.96×10^{10}	10000
MPN(MPN100 ml^{-1})	2.64×10^2	6.86×10^3	4.96×10^3	5.63×10^4	7.69×10^5	6.38×10^6	5000

Least square mean analysis
[a]BIS- Bureau of Indian standard
[b]- Not defined in standard
[c]BDL- Below detection limit

as also reported by Chandra et al. (2009).

The ANOVA showed that 25% to 100% concentrations of distillery effluent showed significant ($P<0.05$) changes in OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, TKN, PO_4^{3-}, SO_4^{2-}, Zn, Cd, Cu, Cr and Mn of the soil (Table 2). More significant ($P<0.001$) changes were also recorded in OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, TKN, PO_4^{3-}, SO_4^{2-}, Zn, Cd, Cu, Cr and Mn, with 10% to 100% concentration of distillery effluent. Non significant ($P>0.05$) changes were found in moisture content, WHC and BD after distillery effluent irrigation (Table 2). The coefficient of correlation (r-value) on soil characteristics reveal EC, pH OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, TKN, PO_4^{3-}, SO_4^{2-}, Zn, Cd, Cu, Mn and Cr, and positively correlate with all distillery effluent concentrations (Table 3). WHC and BD, showed their negative correlation with all concentrations of distillery effluent (Table 3). Moreover, the enrichment factor indicates the enrichment of heavy metals in the soil and were found in order of Zn>Mn>Cr>Cd>Cu after irrigation with distillery effluent (Figure1).

Effect on seed germination of *V. faba*

Most seed germination of *V. faba* was observed with control, while the least were noted with 100% concentration of distillery effluent (Figure 2). The seed germination of *V. faba* were found to negatively correlate ($r = -0.99$) with distillery effluent. The maximum Relative Toxicity (RT) against seed germination of *V. faba*, were recorded with 100% concentration of distillery effluent (Figure 3). The RT were observed to positively correlate ($r = +0.63$) with different concentrations of distillery effluent. This type of germination pattern of *V. faba* is likely due to the presence of toxicants in higher concentration of effluent, which may inhibit the germination at higher concentrations. In addition, the availability uptake and leaching of nutrients is greatly influenced by a number of physico-chemical factors. Among various physico-chemical factors, the pH plays a significant role in the soil. In the acidic soil environment, the availability of basic cations like Ca^{2+}, Mg^{2+}, K^+ and Na^+, becomes lower due to leaching. Thus, the availability of these nutrients decreases as per increase in the acidic character of the soil. However, it directly affects the germination and growth of the crop plants (Kalaiselvi et al., 2008). Similarly, salts are usually the most damaging to young plants, but not necessarily at the time of germination. Although, high salt concentration can slow

Table 2. Physico-chemical characteristics of soil before and after fertigation of *V. faba* with distillery effluent i.e. after crop harvesting of 90 days**.**

Parameter)	**Bore well water (Control)**	**Effluent concentration (%)**					**F-calculated**	**Critical difference**
		10	**25**	**50**	**75**	**100**		
Soil texture	Loamy	Loamy	Loamy	Loamy	Loamy	Loamy	-	-
Soil moisture (%	60.44	58.47	56.36	52.80	50.86	48.50	1.22ns	7.42
WHC (%)	45.24	44.63	42.58	41.75	40.82	39.64	1.16ns	8.13
BD (gm cm^{-3})	1.42	1.42	1.41	1.41	1.40	1.40	0.52ns	0.2
EC (dS m^{-1})	2.06	2.56a	2.94a	3.64a	4.68a	5.86a	33.08**	0.31
pH	7.51	7.40	7.03	6.66a	6.31a	6.27a	6.1ns	0.62
OC(mg Kg^{-1})	0.42	1.92a	4.26a	8.63a	12.86a	16.98a	196.44***	1.6
Na^{+} (mg Kg^{-1})	18.81	25.47	38.96a	44.67a	64.75a	88.69a	45.75**	7.4
K^{+}(mg Kg^{-1})	155.34	164.88a	224.76a	264.80a	286.30a	296.74a	83.96**	9.36
Ca^{2+} (mg Kg^{-1})	15.36	26.80a	138.56a	154.30a	194.75a	224.70a	612.75**	6.85
Mg^{2+}(mg Kg^{-1})	1.70	4.53	22.40a	34.66a	48.76a	76.40a	26.34**	4.48
TKN(mg Kg^{-1})	32.21	74.20a	142.66a	274.52a	316.86a	458.94a	4536.52***	7.68
PO_4^{3-} (mg Kg^{-1})	53.00	58.36a	74.50a	132.45a	186.56a	218.55a	712.60**	7.42
SO_4^{2-} (mg Kg^{-1})	74.37	88.40a	112.47a	153.64a	176.64a	196.80a	44.76**	9.23
Fe^{2+}(mg Kg^{-1})	2.65	4.26	6.48a	8.40a	10.36a	12.48a	30.12**	1.74
Cd (mg Kg^{-1})	0.74	1.44a	2.74a	3.18a	3.76a	5.42a	39.63***	0.53
Cr (mg Kg^{-1})	0.56	0.52a	0.86a	1.59a	2.98a	4.67a	3.03***	0.018
Cu (mg Kg^{-1})	2.48	2.28	4.41a	7.20a	8.36a	12.45a	125.36***	0.96
Mn (mg Kg^{-1})	0.84	1.24	2.30a	3.86a	5.47a	8.96a	15.42***	0.09
Zn (mg Kg^{-1})	0.62	2.14	3.75a	5.12a	7.43a	9.84a	54.26***	0.27

Least square mean analysis; Significant F -***P > 0.1%, **P >1% level,*P> 5% level; ns-non significant; a- significantly different to the control

seed germination by several days or completely inhibit it (Pandey et al., 2008). Soluble salts move readily with water and evaporation transfers salts to the soil surface where they accumulate, and make the soil surface harden. As a result, there might be delay in seed germination (Kumar and Chopra, 2012).

Effect on vegetative growth of *V. faba*

At vegetative growth (45 days after planting) of *V. faba*, the maximum shoot length, root length, chlorophyll content, LAI and dry weight of *V. faba* were noted with 25% concentration of distillery effluent (Table 4). Distillery effluent irrigation had significant ($P<0.05$) effect on shoot length of *V. faba*, whereas root length, chlorophyll content, LAI and dry weight of *V. faba* noted insignificant ($P>0.05$) affected after distillery effluent irrigation. At vegetative growth stage, the performance of *V. faba* was gradually increased from 10% to 25% concentration of distillery effluent, and decreased when the effluent concentrations increased from 50% to 100%. The agronomical parameters at vegetative growth stage showed insignificant positive correlation with distillery effluent concentrations (Table 5). These observations were in agreement with the earlier reported results by Kannan and Upreti (2008) and Mohamed and Ebead (2013). Tharakeshwari and Jagannath (2011a) also reported that 25% concentration of distillery effluent were found to be beneficial to the growth of shoot and root in *V. radiate* (L.) and *V. unguiculata* (L.) plants as compared to control; indicating the enhancing influence of

Table3. Coefficient of correlation (r) between distillery effluent and soil characteristics.

Distillery effluent /soil characteristics	r -value
Distillery effluent versus soil WHC	-0.94
Distillery effluent versus soil BD	-0.96
Distillery effluent versus soil EC	+0.91
Distillery effluent versus soil pH	+0.89
Distillery effluent versus soil OC	+0.89
Distillery effluent versus soil Na^+	+0.95
Distillery effluent versus soil K^+	+0.96
Distillery effluent versus soil Ca^{2+}	+0.98
Distillery effluent versus soil Mg^{2+}	+0.97
Distillery effluent versus soil TKN	+0.99
Distillery effluent versus soil PO_4^{3-}	+0.95
Distillery effluent versus soil SO_4^{2-}	+0.99
Distillery effluent versus soil Fe^{2+}	+0.88
Distillery effluent versus soil Cd	+0.87
Distillery effluent versus soil Cr	+0.87
Distillery effluent versus soil Cu	+0.96
Distillery effluent versus soil Mn	+0.97
Distillery effluent versus soil Zn	+0.97

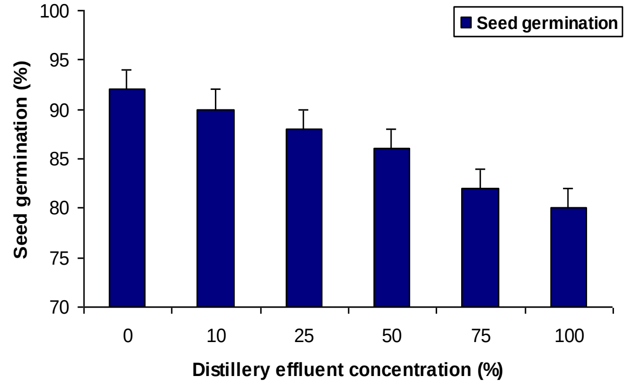

Figure 2. Seed germination of V. *faba* after fertigation with distillery effluent. Error bars are standard error of the mean.

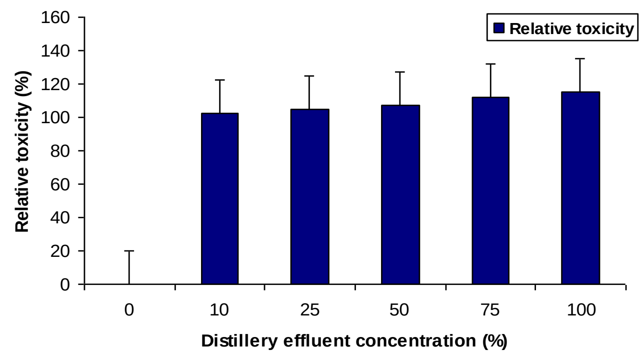

Figure 3: Relative toxicity of distillery effluent against seed germination of *V. faba.* Error bars are standard error of the mean.

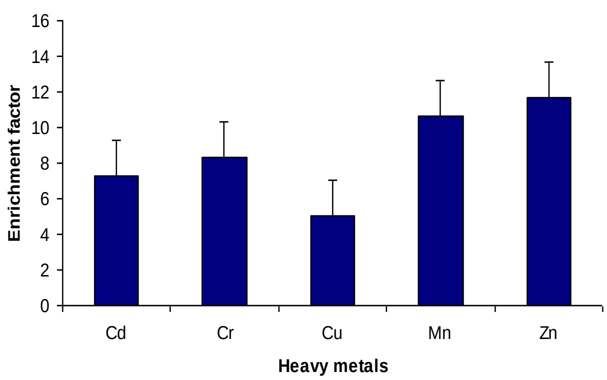

Figure1: Enrichment factor of heavy metals in soil after fertigation with distillery effluent. Error bars are standard error of the mean.

plant nutrients present in the effluent. Bharagava et al. (2008) also found that post distillery effluent irrigation increases the chlorophyll and protein contents in Indian mustard plants (*Brassica nigra* L.) at the lower concentrations (25% and 50%) of distillery effluent followed by a decrease at higher concentrations (75% and 100%) of distillery effluent as compared to their respective controls.

At vegetative growth stage, the growth of *V. faba* was decreased at higher concentration of distillery effluent. The higher EC is the indication of higher salt content in higher concentrations of distillery effluent. The higher salt content acts as a limiting factor for the seed germination and vegetative growth. Thus, the higher EC found to be responsible for the inhibition in plant growth. These findings suggest a link between EC and plant growth as has earlier been reported by Kumar and Chopra (2012). The chlorophyll content of *V. faba* were observed higher with 25% distillery effluent, and this is likely due to the presence of significant content of Fe, Mg and Mn in the distillery effluent, which is associated with the synthesis of chlorophyll in plants. The deficiency of Fe, Mn and Mg in plants, causes significant changes in plant metabolism, and induces chlorosis and necrosis, early leaf fall and low reutilization as earlier reported by Porra (2002).

Effect on flowering of *V. faba*

At flowering stage (60 days after planting) the number of flowers of *V. faba* were affected insignificantly ($P>0.05$) after distillery effluent irrigation (Table 4). Most flowers of *V. faba* were observed with 25% concentration of distillery effluent. The number of flowers of *V. faba* was decreased When the effluent concentration increased from 50% to

Table 4. Effect of distillery effluent fertigation on agronomical characteristics of *V. faba*.

Effluent concentration (%)	Vegetative growth stage (45 DAP)					Flowering (60 DAP) and Maturity stage (90 DAP)			
	Shoot length (cm)	Root length (cm)	Chlorophyll content	LAI	Dry weight	Number of flowers	Number of pods	Crop yield	HI
0 (control)	74.85	15.42	3.64	3.84	55.67	36	32	175.36	314.99
10	78.65	18.69	3.86	3.92	58.86	42	40	185.66	315.42
25	106.45a	20.36	4.36	4.12	64.96	54	51	234.75a	361.37a
50	98.78a	19.86	4.23	4.02	62.36	50	47	224.88a	360.61a
75	87.66a	18.47	4.12	3.96	60.12	48	44	212.36a	353.22a
100	80.48	16.77	3.74	3.88	57.75	44	38	196.84	340.84
CD	9.63	1.86	0.12	0.23	2.67	3.84	3.62	11.86	12.75
F-calculated	63.47*	2.36ns	1.24ns	1.47ns	3.14ns	3.21ns	3.11ns	62.37*	52.44*

Least square mean analysis; Significant F -*P> 5% level; ns-non significant; a- significantly different to the control; CD-Critical difference; DAP-Days after planting.

Table 5. Coefficient of correlation (r) between distillery effluent and agronomical characteristics of *V. faba*.

Distillery effluent / *V. faba*	r - value
Distillery effluent versus shoot length	+0.07
Distillery effluent versus root length	+0.01
Distillery effluent versus chlorophyll content	+0.07
Distillery effluent versus LAI	+0.12
Distillery effluent versus dry weight	+0.07
Distillery effluent versus number of flowers/plant	+0.32
Distillery effluent versus number of pods/plant	+0.15
Distillery effluent versus crop yield/plant	+0.28
Distillery effluent versus harvest index	+0.51
Distillery effluent versus Cd	+0.92
Distillery effluent versus Cr	+0.90
Distillery effluent versus Cu	+0.96
Distillery effluent versus Mn	+0.97
Distillery effluent versus Zn	+0.97

100%, and this insignificantly positively correlates with distillery effluent concentrations (Table 5). 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. The maximum flowering of *V. faba* were observed with the 25% concentration of distillery effluent and this might be due to the 25% concentration of N and K. Furthermore, N and K prevent flower abortion so pod formation occurs (El-Naggar, 2005).

Effect on Maturity stage of *V. faba*

At maturity stage (90 days after planting) the maximum number of pods/plant, crop yield and harvest index of *V. faba* was with 25% concentration of distillery effluent (Table 4). Crop yield and HI of *V. faba* were recorded to be significantly (P<0.05) affected with distillery effluent fertigation. Number of pods/plant of *V. faba* were noted insignificantly (P>0.05) different with effluent fertigation. HI of *V. faba* significantly positively correlates with distillery effluent fertigation (Table 5).

The role of K, Fe, Mg and Mn at maturity is important and associated with synthesis of chlorophyll and enhances formation of pods at harvest (Porra, 2002). The K, Fe, Mg and Mn contents could benefit pod formation and yield of the plant, as it does for soybean (*Glycine max* L.) as reported by Hati et al. (2007). The 25% distillery effluent favoured pod formation and crop yield of *V. faba.* This is likely due to presence of K, Fe, Mg and Mn contents in 25% and 50% distillery effluent; higher distillery effluent concentrations lowered pod formation and crop yield of *V. faba*.

Translocation of heavy metals in *V. faba*

The metals content in various plant parts of *V. faba* were increased as per distillery effluent concentration increased.

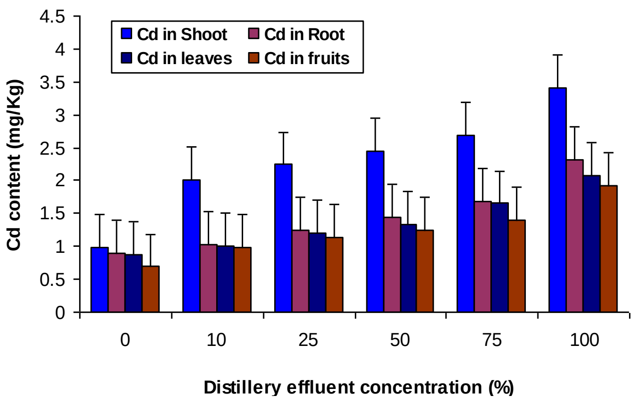

Figure 4: Translocation of Cd in various parts of *V. faba* after fertigation with distillery effluent. Error bars are standard error of the mean.

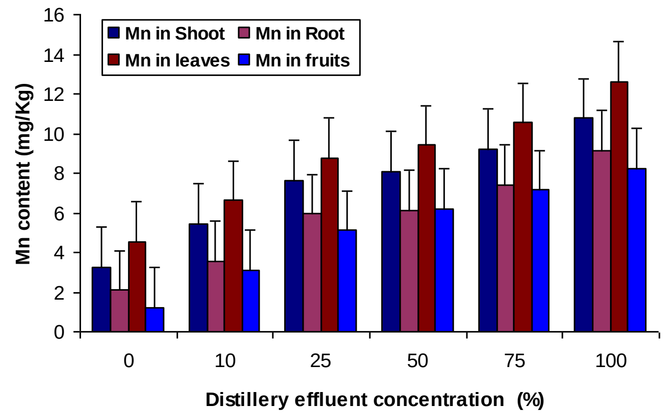

Figure 7: Translocation of Mn in various parts of *V. faba* after fertigation with distillery effluent. Error bars are are standard error of the mean.

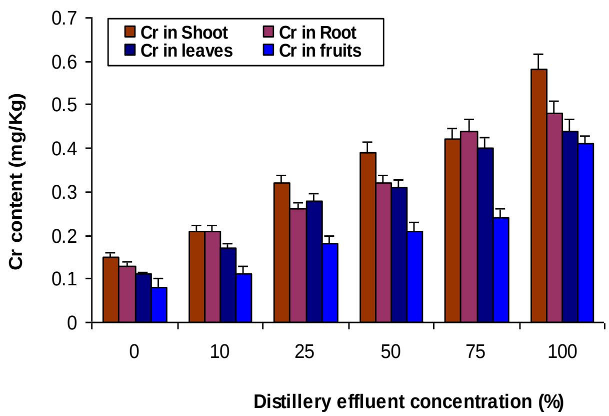

Figure 5: Translocation of Cr in various parts of *V. faba* after fertigation with distillery effluent. Error bars are standard error of the mean.

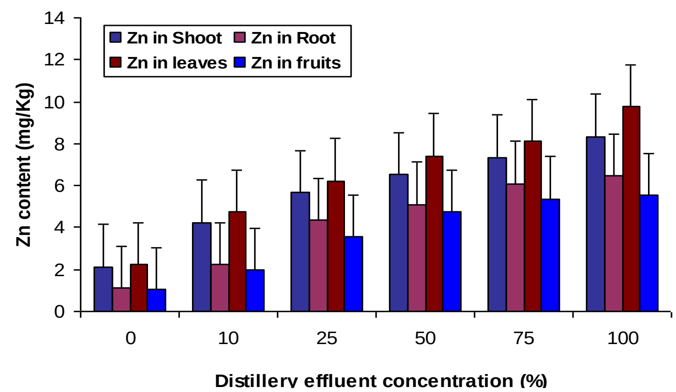

Figure 8: Translocation of Zn in various parts of *V. faba* after fertigation with distillery effluent. Error bars are standard error of the mean.

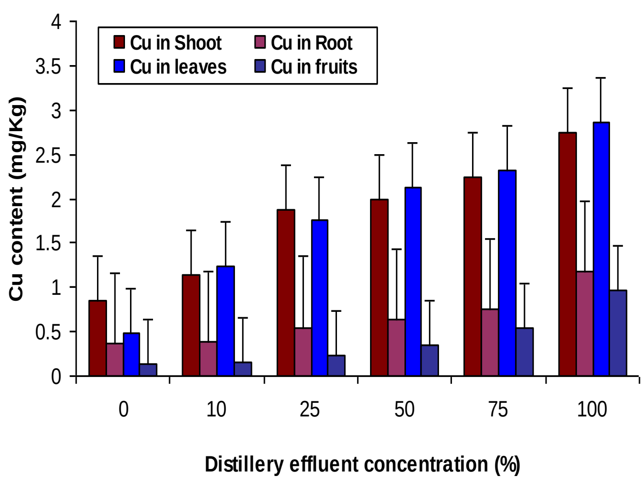

Figure 6: Translocation of Cu in various parts of *V. faba* after fertigation with distillery effluent. Error bars are standard error of the mean.

The translocation of various heavy metals in different parts of *V. faba* was in order of leaves>shoot>root> fruits for Cu, Mn and Zn, root>shoot>leaves>fruit for Cd and shoot>root>leaves>fruit for Cr in *V. faba* after distillery effluent irrigation (Figures 4-8). The maximum enrichment factor were recorded for Cu (4.96) while the minimum was for Cd (2.8) in *V. faba* and it was found in order of Cu>Zn>Mn>Cr>Cd in *V. faba* after irrigation with distillery effluent (Figure 9). Chandra et al. (2009) reported higher content of metals (Cu, Cd, Cr, Zn, Fe, Ni, Mn, and Pb) in wheat and mustard plants irrigated with mixed distillery and tannery effluents.

Effect on biochemical constituents of *V. faba*

The maximum crude proteins, crude fiber and crude carbohydrates of *V. faba* were recorded with 25% concentration of distillery effluent (Figure 10). Content of crude proteins (r = +0.40), crude fiber (r = +0.35) and crude

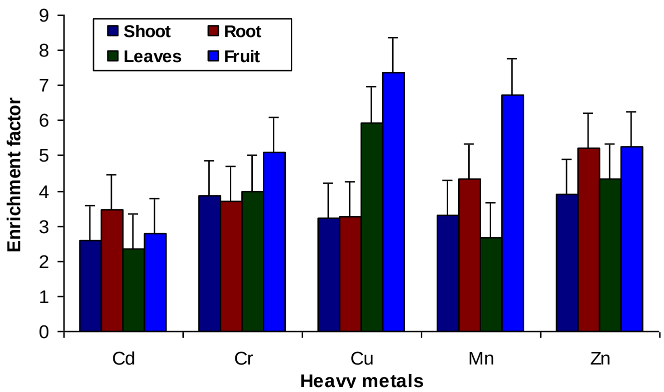

Figure 9: Enrichment factor of heavy metals in various parts of *V. faba* after fertigation with distillery effluent. Error bars are standard error of the mean

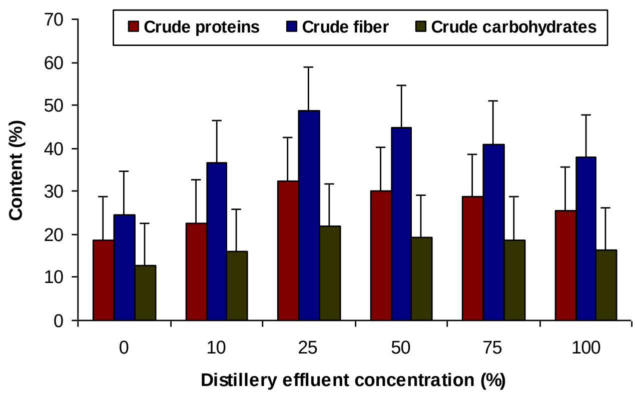

Figure 10: Content of crude proteins, crude fiber and crude carbohydrates in *V. faba* after fertigation with distillery effluent. Error bars are standard error of the mean

carbohydrates (r = +0.27) of *V. faba* were noted to positively correlate with distillery effluent concentration. The changes in biochemical constituents were in accordance with Sukanya and Meli (2004), which reported that the nutrients quality of wheat was decreased when the concentration of distillery effluent was increased.

Conclusions

It was concluded from this research that distillery effluent is rich in plant nutrients and heavy metals. Fertigation with distillery effluent added the nutrients as well as heavy metals in the soil environment. Distillery effluent fertigation significantly ($P<0.01$) increased the EC, pH, Cl^-, 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. The agronomical performance of *V. faba* was gradually increased at lower concentration i.e. from 10% to 25% concentration of distillery effluent and decreased at higher concentrations i.e. 50% to 100% concentration of distillery effluent. It might be due to certain growth stimulants and inhabitants present in distillery effluent that are responsible for this type of growth performance. The maximum agronomical growth of *V. faba* was observed with 25% concentration of distillery effluent. The enrichment factor of heavy metals was in order of Zn>Mn>Cr>Cd>Cu for soil and Cu>Zn>Mn>Cr>Cd in *V. faba* after fertigation with distillery effluent. The translocation of various heavy metals- plant part specific - were found in order of leaves>shoot>root> fruits for Cu, Mn and Zn, root>shoot>leaves>fruit for Cd and shoot>root>leaves>fruit for Cr in *V. faba* after distillery effluent irrigation. Thus the effluent can be used after dilution (up to 25%) for maximum yield of *V. faba*. Further studies are required on changes in soil and biochemical components of *V. faba* after distillery effluent fertigation.

ACKNOWLEDGEMENTS

The University Grants Commission, New Delhi, India is acknowledged for providing the financial support in the form of UGC research fellowship to the corresponding author.

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