Applied Biological Research 23(1): 76-86; (2021)
DOI: 10.5958/0974-4517.2021.00010.0

IMPACT OF COW MANURE APPLICATION AS SOIL AMENDMENT ON ESSENTIAL OIL COMPOSITION AND METAL CONTENTS IN FENNEL (*Foeniculum vulgare* Mill)

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(Received 10 August, 2020; accepted 29 December, 2020)

ABSTRACT

The natural resources and agriculture ecosystem are adversely damaged due to the fast buildup of toxic pollutants such as radionuclide, organic contaminants and heavy metals emitted from industries and other anthropogenic activities. A polyhouse experiment was conducted to assess the capability of fennel (*Foeniculum vulgare*) to remediate a metal contaminated soil, collected from Moradabad (a hub of electroplating industries), by the application of different levels of cow manure. Metal analysis of root and aerial parts was performed using AAS and essential oil composition by GC and GC/MS. The results revealed that the use of cow manure at 52 t ha^{-1} gave highest plant growth and oil yield. Limonene, *trans*-anethole and dillapiole were the major constituents among all the amendments. The highest content of *trans*-anethole (56.05%) was noticed at 52 t ha^{-1}. The addition of cow manure reduced Zn, Ni, Cu and Cr concentration in plant tissues. Maximum reduction was found at 104 t ha^{-1}. Cr showed highest BCF at 0 t ha^{-1}, while Cu showed lowest BCF at 104 t ha^{-1}. Therefore, cow manure use in the metal contaminated soils had favourable impact on the growth characters and essential oil composition of fennel.

Keywords: Cow manure, essential oil, *Foeniculum vulgare*, heavy metals

INTRODUCTION

Heavy metal toxicity and their bioaccumulation in food chain is a major environmental challenge. Municipal wastes, sewage sludge, pesticides, fertilizers, fossil fuel burning, mining and smelting of metallic ferrous ores are the leading sources of contamination (Peng *et al.,* 2006). Nickel (Ni), chromium (Cr), cadmium (Cd), lead (Pb), copper (Cu), mercury (Hg), and zinc (Zn) are the most common heavy metal contaminants (Lasat, 2002).

Worldwide over 2000 plant species belonging to 60 families are presently used in the production of essential oils (Van de Braak and Leijten, 1999). Fennel (*Foeniculum vulgare* Mill.) belongs to the family Apiaceae (Umbelliferae) and is a biennial aromatic medicinal plant, native to the Mediterranean Sea and near Eastern regions (Senatore *et al.,* 2013). It has been exported from country to country for years due to its culinary values (Barros *et al.,* 2010). The other major uses of essential oil of fennel are flavouring food preparations and use in perfumery industries. Its seeds are used in sauces, savoury formulations, liqueurs, confectionery, pastries, pickles, meat dishes, etc.

(Bhati *et al.*, 1988). Antidiabetic, anti-inflammatory, anti-tumour, and many other activities of fennel plant have been reported by Khazaei *et al.* (2011). The flavour of fennel depends upon its principal constituents: anethole, fenchone and estragole; and the quantities of these ingredients alter according to the phonological state and origin of fennel (Diaaz-Maroto *et al.*, 2006).

Compost fertilizer is safe for the environment as well as for human health and has a positive effect on various growth characters of plants. Many factors such as soil selection, fertilizer management and various agro-techniques affect the productivity and quality of essential oil producing crops (Darzi *et al.*, 2009). Mycorrhiza, phosphate and vermicompost amendments increased the anethole content of sweet fennel and decreased the content of limonene (Younesian *et al.*, 2013).

Monitoring and assessment of heavy metal content in the different crops including vegetables and aromatic plants as well as changes in heavy metal concentration with the application of manure have limited published data (Mohamed and Abdu, 2004; Kumpiene *et al.*, 2008; Moradi *et al.*, 2009). The essential oil yield of fennel increased by the application of chicken manure (Mohamed and Abdu, 2004). Enhancement in the essential oil yield by compost treatment has been documented from Iran (Moradi *et al.*, 2009). Kumpiene *et al.* (2008) have observed that organic amendments stabilize metals in soil and reduce metal availability and mobility as well as increase the content of metals in plant shoots. The present study was aimed to assess the effect of manure amendments on the heavy metal (Cu, Zn, Ni and Cr) accumulation and determine the essential oil composition of fennel.

MATERIALS AND METHODS

Soil collection

The composite surface soil samples (0-20 cm) were collected from a highly metal polluted site in Moradabad (Karulanala) where electroplating industrial wastes were disposed. The seeds of fennel (*Foeniculum vulgare*) were purchased from the Vegetable Research Centre (VRC), Pantnagar Uttatakhand (India) and cow manure was collected from Nainital (India). The identification of the plant was authenticated by the Botanical Survey of India, Dehradun, Uttarakhand (India).

Pot experiment

Air-dried grounded soil samples were treated with four applications of cow manure (0, 26, 52 and 104 t ha^{-1}). Each treatment was replicated four times. The cow manure amended soils were incubated for one month and the initial moisture content was maintained during incubation period by watering it regularly (27°C temperature and 65% relative humidity). Then 3 kg amended soils were placed in the pots of 5 kg capacity (25 cm dia.). The pots had free draining facility. A separate dish was also used for placing each pot, resulting the prevention of leaching of heavy metals. Thirty healthy seeds of fennel were sown in each pot. The plants were watered regularly and first cutting of fennel was done after 30 days and the contents of heavy metals (Cu, Zn, Cr and Ni) was determined in soil and aerial parts (1^{st} cutting). The thinning of plants was also done at the same time and 10 plants pot^{-1} were maintained. The crop was harvested after 90 days of growth (2^{nd} cutting) and metal accumulation and essential oil contents were determined.

Assessment of physicochemical properties of soil and manure and heavy metal analysis

The pH and EC of soil and manure samples were measured using an electric shaker for half an hour in soil/manure-water suspension (1:2) (Jackson, 1958). Heavy metal analysis was performed using 2 g composite soil/manure samples digested with 8 mL of aqua regia on a sand bath for 2 h (Lokeshwari and Chandrappa, 2006). The texture of soil was determined by pipette method, (Kilmer and Alexander, 1949). Other physicochemical properties such as organic carbon, nitrogen, potassium, phosphorus and heavy metals (Zn, Ni, Cu and Cr) were measured in soil/manure samples as per standard methods (Jackson, 1958). The metal content in oil and water samples were determined using nitric acid and perchloric acid as per the method of Zheljakov and Nielson (1996a).

Isolation, analysis and identification of essential oils

The hydrodistillation of fresh harvested plant materials was performed on Clevenger apparatus for 3 h. The hydrodistilled oil was dried over anhydrous sodium sulphate (Abdellatif and Hassani, 2015) and stored at 4°C in a BOD incubator before use. The oil samples were analyzed by using a Shimadzu 2010 gas chromatograph (GC) in which the oven temperatures were programmed at 80, 210 and 300°C with a hold time of 2, 5 and 15 min, respectively. Injection temperature was 270°C with 0.2 µL injection volume (manual injection) and 1:20 spilt ratio. FID was maintained at 280°C temperature. Carrier gas was N_2 with Rtx-5 column (30 m × 0.25 mm × 0.25 µm film thickness). The essential oil components were analyzed by gas chromatography-mass spectrometry (GC-MS) which was carried out on 2010 GC coupled with Shimadzu Q P 2010 plus with Rtx-5 fused silica capillary column under similar conditions as those of GC. Mass range for MS was 40-600 amu. The temperature for Ramp 1 and Ramp 2 was 3°C min^{-1} and 20°C min^{-1}, respectively. Ion source temperature was 200°C with EI (70 eV) mode and 1 mL min^{-1} flow rate. Carrier gas was helium. The components of essential oils were identified on the basis of retention index (RI, determined with reference to homologous series of n-alkanes; C_9-C_{33}, (Polyscience Corp., Niles IL) under identical experimental conditions), co-injection with standards and MS Library search (NIST: NIH version 2.1 and WILEY: 7th edition) and by comparing with the mass spectrometry literature (Adams, 2007). The relative content of individual components was calculated on the basis of GC FID response without using correction factor.

Data analysis

The analysis of selected metals (Zn, Cr, Cu and Ni) in fennel was done by using data analysis formulae with respect to bioaccumulation factor (BCF) and translocation factor (TLF) (Ma *et al.*, 2001).

$$\text{Bioconcentration factor (BCF)} = \frac{\text{Metal content in roots}}{\text{Metal content in soil}}$$

$$\text{Translocation factor (TLF)} = \frac{\text{Metal content in aerial plant parts}}{\text{Metal content in plant roots}}$$

Statistical analysis

The results were subjected to one-way analysis of variance (ANOVA) to assess the significant difference between means at $p < 0.05$ level. Correlation coefficients were calculated at probability levels of $p < 0.01$ and $p < 0.05$ (Punetha *et al.*, 2019a) using SPSS 16.0.

RESULTS AND DISCUSSION

Physicochemical properties of soil and cow manure

The physicochemical properties of Karulanala soil are given in Table 1. Karulanala soil, collected from Moradabad district, was an alkaline soil with a pH value of 7.89. Soil pH affects not only the metal bioavailability, but also metal uptake into the roots. This effect appears to be metal specific. The soil had electrical conductivity of 0.4 dS m^{-1}, organic carbon 0.9% and organic matter 1.5%. Mechanical analysis of soil revealed it to be sandy clay loam in texturewith 52% sand, 34% silt and 14% clay. The N, P and K content was 0.224, 0.005, 0.008%, respectively. Karulanala soil had high concentration of Zn (544.00 mg kg^{-1}), Cu (643.21 mg kg^{-1}) and Cr (131.00 mg kg^{-1}). Similarly, cow manure used in present study had 7.6 pH, 1.2 dS m^{-1} EC, 7.3% organic carbon and 12.6% organic matter content. The cow manure had optimum amounts of macronutrients (2.03% N, 2.95% P and 2.16% K) and micronutrients (235.0 mg kg^{-1}; Zn, 4.50 mg kg^{-1} Cu and 3.55 mg kg^{-1} Ni). The vegetables grown at the site reportedly have high metal accumulation (Punetha *et al.*, 2015).

Table 1: The physicochemical properties of soil and cow manure

Material tested	pH	EC ($dS\ m^{-1}$)	Organic carbon (%)	Organic matter (%)	Mechanical analysis (%)			Textural class
					Sand	Silt	Clay	
Cow manure	7.6 ± 0.5	1.2 ± 0.2	7.3 ± 0.2	12.6 ± 0.5	-	-	-	-
Karulanala soil	7.9 ± 0.3	0.4 ± 0.2	0.9 ± 0.1	1.5 ± 0.4	52	34	14	Sandy clay loam

	Macronutrients (%)			Micronutrients ($mg\ kg^{-1}$)			
	Total N	P_2O_5	K_2O	Zn	Ni	Cu	Cr
Cow manure	2.03 ± 0.10	2.95 ± 0.20	2.16 ± 0.20	235.0 ± 5.3	3.6 ± 0.1	4.5 ± 0.4	-
Karulanala soil	0.22 ± 0.00	0.004 ± 0.0	0.01 ± 0.0	544.0 ± 4.6	35.3 ± 0.2	643.2 ± 1.3	131.0 ± 4.1

The values given are means of three replications ± standard deviation

Growth of fennel

Increase in the cow manure level not only effectively increased the macronutrient (N, P, and K) content but also the plant height. Height of plant was taken after first month and found that 52 t ha^{-1} showed the highest plant height (11 cm) and the lowest (9 cm) at 104 t ha^{-1} amendment. Maximum plant height (49 cm) was obtained with the application of 52 t ha^{-1} concentration and the minimum plant height of 39 cm in 104 t ha^{-1} concentration of cow manure after third month of plant growth. A study also suggested that compost addition to soil can be used to maintain and improve soil structure as its organic matter content can counteract the natural decline in intensively cultivated soils (Lillenberg *et al.*, 2010).

Another key factor responsible for total growth of a crop is fresh plant weight. A significant enhancement in fresh plant weight was seen during different cow manure applications (168.0, 184.2, 255.0 and 207.1 g for 0, 26, 52 and 104 t ha^{-1} amendments, respectively) with maximum fresh weight in 52 t ha^{-1} amendments and minimum in control treatment (Table 2). Improvement in the soil physical properties (density, compaction and aggregation, root penetration, water holding capacity and water infiltration) by organic matter amendments has also been reported (Stewart *et al.*, 2000). The nutrient supplemented through organic sources influenced the growth, yield and quality of aromatic plants (Kumar *et al.*, 2015; Punetha *et al.*, 2019a).

The weight of seeds was also used to find out the ultimate growth and yield response of a crop. The fresh seed weight was significantly influenced by the application of different cow manure amendments. The maximum seeds weight (129.8 g) was obtained at 52 t ha^{-1} level and minimum (40.2 g) in control (0 t ha^{-1}). These results are in agreement with Salarazai *et al.* (2005) who showed that there was significant impact of the nitrogen levels on the growth and yield of fennel plant.

Oil productivity

The increase in the quantity of cow manure applied to the soil resulted in a significant increase in the plant oil yield. Our results are in agreement with Kapoor *et al.* (2004) who reported a positive effect of organic fertilizers addition to soil on the essential oil yield of fennel. Maximum oil yield of 0.4% (w/w) was obtained by using 52 t manure ha^{-1}, while minimum oil yield of 0.2% (w/w) was obtained in control (Table 2). The increase in the oil yield with 52 t manure ha^{-1} (50% increase over

Table 2: Effect of cow manure amendments on the growth of fennel grown in Karulanala soil

Amendments ($t\ ha^{-1}$)	Height (cm)		Oil yield (% w/w)	Fresh weight ($g\ 10\ pot^{-1}$)	
	I month	III month		whole plant	fennel seeds
0	$10^{a} \pm 2.6$	$40^{a} \pm 4.6$	$0.2^{a} \pm 0.0$	$168.0^{a} \pm 3.6$	$40.2^{a} \pm 2.5$
26	$10^{a} \pm 1.7$	$42^{a,b} \pm 3.6$	$0.2^{a} \pm 0.0$	$184.2^{b} \pm 3.9$	$51.2^{b} \pm 1.2$
52	$11^{a} \pm 1.0$	$49^{c} \pm 2.0$	$0.4^{b} \pm 0.0$	$255.0^{c} \pm 7.8$	$129.8^{c} \pm 3.4$
104	$9^{a} \pm 1.7$	$39^{a} \pm 4.6$	$0.2^{a} \pm 0.0$	$207.1^{d} \pm 2.8$	$103.6^{d} \pm 5.6$

The values are the means of three replications ± standard deviation. The mean values superscripted by the same alphabets (a-d) within a column are not significantly different at $p<0.05$ as per to Duncan's test

Table 3: Correlation among metal content, growth parameters and essential oil composition of fennel

Parameters	Zn in aerial parts	Cu in aerial parts	Ni in aerial parts	Cr in aerial parts	Plant height	Plant weight	Seed weight	Oil yield	MH	PP	SH
Zn in aerial parts	1.000	0.981*	0.922	0.985*	-0.186	-0.700	-0.860	-0.336	-0.211	-0.337	0.928
Cu in aerial parts		1.000	0.958*	0.993**	0.007	-0.549	-0.751	-0.149	-0.344	-0.174	0.969*
Ni in aerial parts			1.000	0.974*	0.077	-0.432	-0.614	-0.012	-0.203	-0.220	0.878
Cr in aerial parts				1.000	-0.058	-0.587	-0.764	-0.188	-0.233	-0.273	0.932
Plant height					1.000	0.821	0.616	0.961*	-0.701	0.891	0.137
Plant weight						1.000	0.955*	0.906	-0.306	0.778	-0.452
Seed weight							1.000	0.761	-0.037	0.593	-0.693
Oil yield								1.000	-0.477	0.789	-0.067
MH									1.000	-0.825	-0.557
PP										1.000	0.036
SH											1.000

*Correlation coefficient significant at $p<0.05$; **Correlation coefficient significant at $p\leq0.01$; MH = Monoterpene hydrocarbons; PP = Phenyl propanoids and SH = Sesquiterpene hydrocarbons.

control) might be attributed to the increase in seed weight, number of umbel plant^{-1} and seeds umbel plant^{-1} (Cserni and Sass, 1994). In present study, the oil yield was found positively correlated with plant height (r^2 = 0.961; $p<0.05$) (Table 3).

Metal accumulation by fennel

Many plant species grown in heavy-metal enriched soils accumulate metals in the shoots and these plants can be used to clean up metal-contaminated sites (Peng *et al.,* 2006). Fennel grown in cow manure amended soils showed significant differences in the concentration of all the four metals after first and third month (Table 4). Accumulation of different metals by fennel may be explained as follow:

Zinc: Fennel grown in contaminated soil showed high Zn accumulation in the 2nd cutting (90 days) as compared to the 1st cutting (Table 4). Zinc content in contaminated soil was higher than the permissible limit for both the cuttings (70.3 and 77.5 mg kg^{-1}, respectively). The metal concentration in the plant tissue is a function of metal content in growing media (Grifferty and Barrington, 2000; Cui *et al.,* 2004). Increase in the quantity of manure led to a decrease in Zn content in both the cuttings and this decrease was higher in 104 t ha^{-1} cow manure amended soil for both the cuttings. Zinc content tended to decrease in the aerial parts as well as in roots, when treated with different amendments. The order of heavy metals in shoots of contaminated and manure amended contaminated soil was as follow;

70.3 (0 t ha^{-1}) > 63.7 (26 t ha^{-1}) >49.9 (52 t ha^{-1}) > 41.9 (104 t ha^{-1}) (1st cutting in mg kg^{-1})

77.5 (0 t ha^{-1}) > 68.9 (26 t ha^{-1}) > 57.3 (52 t ha^{-1}) > 40.0 (104 t ha^{-1}) (2nd cutting in mg kg^{-1})

Roots had the following Zn accumulation order:

84.8 (0 t ha^{-1}) > 60.1 (26 t ha^{-1}) > 48.6 (52 t ha^{-1}) > 42.9 (104 t ha^{-1}) in mg kg^{-1}

Bioconcentration factor (BCF) and translocation factor (TLF) in fennel for Zn metal was as follow;

0.89 (0 t ha^{-1}) > 0.66 (26 t ha^{-1}) > 0.51 (52 t ha^{-1}) > 0.42 (104 t ha^{-1}) (BCF)

1.18 (52 t ha^{-1}) > 1.16 (104 t ha^{-1}) > 1.15 (26 t ha^{-1}) >0.91 (0 t ha^{-1}) (TLF)

It was seen that BCF and TLF decreased as the amount of cow manure increased in all the amendments which showed that Zn accumulation in roots and shoot was negatively influenced by

Table 4: Effect of cow manure amendments on Zn, Ni, Cu & Cr content in Karulanala soil and fennel (Permissible limit: 50 mg kg^{-1})

Metal	Treatments	Soil (mg kg^{-1})		Aerial parts (mg kg^{-1})		Roots (mg kg^{-1})	BCF (Root/Soil)	TLF (Shoot/Root)
		1st cutting	2nd cutting	1st cutting	2nd cutting			
Zn	A_1	$227.3^a \pm 6.0$	$94.9^a \pm 3.4$	$70.3^a \pm 5.6$	$77.5^a \pm 5.7$	$84.8^a \pm 4.8$	$0.89^a \pm 0.04$	$0.91^a \pm 0.10$
	A_2	$232.8^a \pm 7.7$	$91.1^a \pm 7.6$	$63.7^{a,b} \pm 8.9$	$68.9^a \pm 4.4$	$60.1^b \pm 9.6$	$0.66^b \pm 0.02$	$1.15^b \pm 0.09$
	A_3	$234.6^a \pm 10.9$	$95.8^a \pm 4.7$	$49.9^{b,c} \pm 3.8$	$57.3^b \pm 3.0$	$48.6^c \pm 3.3$	$0.51^c \pm 0.04$	$1.18^b \pm 0.05$
	A_4	$232.3^a \pm 7.1$	$101.7^a \pm 5.3$	$41.9^c \pm 10.0$	$50.0^b \pm 6.2$	$42.9^{c,d} \pm 2.0$	$0.42^d \pm 0.01$	$1.16^b \pm 0.03$
Ni	B_1	$35.2^a \pm 7.7$	$35.3^a \pm 3.9$	$10.2^a \pm 5.7$	$15.3^a \pm 1.7$	$13.6^a \pm 6.3$	$0.39^a \pm 0.01$	$1.12^a \pm 0.27$
	B_2	$37.2^a \pm 5.5$	$37.4^a \pm 7.5$	$9.2^a \pm 3.1$	$12.9^a \pm 3.2$	$11.1^a \pm 2.8$	$0.30^b \pm 0.05$	$1.16^a \pm 0.07$
	B_3	$39.0^a \pm 6.4$	$38.7^a \pm 6.6$	$6.9^a \pm 3.3$	$12.9^a \pm 3.9$	$11.5^a \pm 4.6$	$0.30^b \pm 0.03$	$1.23^a \pm 0.11$
	B_4	$37.2^a \pm 6.8$	$35.4^a \pm 7.0$	$5.1^a \pm 2.3$	$10.7^a \pm 5.1$	$8.7^a \pm 2.8$	$0.25^c \pm 0.10$	$1.23^a \pm 0.14$
Cu	C_1	$566.3^a \pm 5.5$	$316.9^a \pm 6.4$	$40.6^a \pm 4.9$	$35.6^a \pm 6.5$	$41.2^a \pm 8.2$	$0.13^a \pm 0.04$	$0.86^a \pm 0.18$
	C_2	$577.4^a \pm 10.9$	$321.1^a \pm 7.5$	$35.5^a \pm 4.0$	$34.2^a \pm 10.5$	$37.0^a \pm 2.3$	$0.12^a \pm 0.30$	$0.92^a \pm 0.40$
	C_3	$583.1^a \pm 3.0$	$321.7^a \pm 6.6$	$34.2^a \pm 8.5$	$32.8^a \pm 4.0$	$34.9^a \pm 3.6$	$0.11^a \pm 0.01$	$0.94^a \pm 0.23$
	C_4	$600.2^b \pm 3.3$	$324.5^a \pm 7.3$	$30.1^a \pm 6.8$	$30.5^a \pm 4.6$	$31.2^a \pm 7.4$	$0.10^a \pm 0.11$	$0.98^a \pm 0.21$
Cr	D_1	$60.5^a \pm 6.7$	$27.9^a \pm 2.8$	$80.0^a \pm 6.0$	$78.1^a \pm 5.3$	$75.3^a \pm 4.1$	$2.70^a \pm 1.30$	$1.03^a \pm 0.12$
	D_2	$62.7^{a,b} \pm 7.6$	$29.7^a \pm 3.8$	$72.2^{a,b} \pm 3.2$	$71.1^{a,b} \pm 4.9$	$70.9^{a,b} \pm 4.6$	$2.39^b \pm 0.07$	$1.00^a \pm 0.27$
	D_3	$63.5^{a,b} \pm 2.5$	$28.6^a \pm 8.1$	$65.4^b \pm 4.3$	$66.9^{a,b} \pm 9.2$	$65.1^{b,c} \pm 3.6$	$2.28^c \pm 0.06$	$1.05^a \pm 0.06$
	D_4	$73.0^b \pm 6.5$	$26.4^a \pm 5.3$	$63.1^b \pm 4.9$	$60.0^b \pm 6.2$	$58.2^c \pm 3.6$	$2.21^d \pm 0.12$	$1.03^a \pm 0.12$

A_1, A_2, A_3 and A_4, B_1, B_2, B_3 and B_4, C_1, C_2, C_3 and C_4, and D_1, D_2, D_3 and D_4 are the samples taken from 0, 26, 52 and 104 t ha^{-1} amended soil; BCF = Bioconcentration factor; TLF = Translocation factor.
The means ($\pm$ standard deviation) superscripted by the same alphabet (a-c) at within a column do not significantly differ at $p<0.05$ according to the Duncan's test.

the level of manure addition. TLF was higher than BCF, which showed that Zn was transferred more more to the aerial parts than retained in the roots. These results were similar as to those of Diaz-Barrientos *et al.* (2003), who showed that the application of manure might decrease the availability of the metal in Zn contaminated soils as basic organic matter may increase the soil pH.

Nickel: Nickel accumulation in fennel was more in 2nd cutting as compared to the 1st cutting. Manure addition led to decrease in Ni content in fennel roots and aerial parts and this decrease was better expressed in case of 104 t cow manure ha^{-1} (10.2 to 5.1 and 15.3 to 10.7 mg kg^{-1} in the 1st and 2nd cuttings, respectively) [Table 4].

BCF values in the present study were below 1 in all the treatments that showed the limited ability of fennel to accumulate Ni in roots from soil. BCF values decreased continuously with manure amendments and these results were consistent with those of Singh *et al.* (2010) who correlated BCF values with the lower availability of metal due to manure application. The order of bioconcentration factor and translocation factor in fennel for Ni metal was as follows:

$$0.39\ (0\ \text{t ha}^{-1}) > 0.30\ (26\ \text{t ha}^{-1}) = 0.30\ (52\ \text{t ha}^{-1}) > 0.25\ (104\ \text{t ha}^{-1})\ (\text{BCF})$$

$$1.23\ (104\ \text{t ha}^{-1}) = 1.23\ (52\ \text{t ha}^{-1}) > 1.16\ (26\ \text{t ha}^{-1}) > 1.12\ (0\ \text{t ha}^{-1})\ (\text{TLF})$$

Our results showed that all the contaminated and amended contaminated soil samples had Ni content above the limit (1.5 mg kg^{-1}) as reported by Awasthi (2000).

Copper: Copper is one of the least mobile heavy metals at any pH and organic matter is the main factor controlling Cu retention. The Cu content was highest in the control plant (40.6 and 35.6 mg kg^{-1} in 1st and 2nd cuttings, respectively) and lowest in the highest cow manure amendment (104 t ha^{-1}) in both the cuttings (30.1 and 30.5 mg kg^{-1} in 1st and 2nd cuttings, respectively) [Table 4]. The copper accumulation was higher in the first cutting. Bioconcentration factor and translocation factor in fennel for Cu metals are as follows:

$$0.13\ (0\ \text{t ha}^{-1}) > 0.12\ (26\ \text{t ha}^{-1}) > 0.11\ (52\ \text{t ha}^{-1}) > 0.10\ (104\ \text{t ha}^{-1})\ (\text{BCF})$$

$$0.98\ (104\ \text{t ha}^{-1}) > 0.94\ (52\ \text{t ha}^{-1}) > 0.92\ (26\ \text{t ha}^{-1}) > 0.86\ (0\ \text{t ha}^{-1})\ (\text{TLF})$$

Bioconcentration and translocation factors revealed a reduction in Cu accumulation in roots and aerial parts due to cow manure application. TLF values were found higher than the BCF values, revealing that the roots contained low Ni content than in aerial parts. The availability of copper reduced because of high affinity of metals to organic matter (Merritt and Erich, 2003). Although Cu in all the soil samples was well above its respective critical limit (Awasthi, 2000), it may not affect the yields due to the high binding with the organic matter content in the soil.

Chromium: Chromium accumulation was found higher in 1st cutting as compared to the 2nd cutting (Table 4). As the soil was amended with cow manure, there was a reduction in Cr accumulation in the aerial parts (in both cuttings) (80.0 to 63.1 mg kg^{-1} in 1st cutting and 78.1 to 60.0 mg kg^{-1} in the 2nd cutting). Cunningham *et al.* (1995) showed that various physical, chemical and biological processes could either immobilize the metal within soil or remove the metal from soil. The BCF and TLF values in fennel for Cr metal showed that there was increase in Cr availability in roots, which revealed that Cr could not be transferred easily from roots to the aerial part of fennel. The results of this study showed that all the contaminated and amended contaminated soil samples had Cr content above the permissible limit (20 mg kg^{-1}) as reported by Awasthi (2000).

Comparison of BCF and TLF among all the tested metals revealed that Cr showed highest BCF at 0 t ha^{-1} and Ni showed highest TLF in 52 and 104 t ha^{-1}, which showed that Cr retains more in roots and Ni prefers to translocate to the aerial parts.

Cr > Zn > Ni > Cu (BCF)

Ni > Zn > Cr > Cu (TLF)

Metal content in extracted water and essential oil samples

Extracted water and essential oil samples had low metal concentration, which was below the detectable limit and our results are in agreement with those of Zheljazkov and Nielsen (1996a,b) and Punetha *et al.* (2019a). It reveals that significant amounts of heavy metals could be removed from the soil through proper disposal of metal contaminated plant residues, while metal-free extracted oils could be safely marketed.

Essential oil composition

A total of 20 compounds with significant variations were identified in fennel which represented 95.7, 95.6, 93.5 and 96.3% of the total oil in cow manure treatments at 0, 26, 52 and 104 t ha^{-1}, respectively (Table 5). *Trans*-anethole (39.87-56.05%), the main secondary metabolite of fennel responsible for the characteristic aroma, was the major constituent in plants of all the treatments. Darzi *et al.* (2009) reported that the application of biofertilizers to the contaminated soil alters the quantity of essential oil constituents in fennel. *Trans*-anethole was reported to be the main component in fennel plant (Negahban *et al.*, 2015; Punetha *et al.*, 2019b). In present study the maximum *trans*-anethole content was observed in 52 t cow manure ha^{-1} soil amendment and minimum in control. Moradi *et al.* (2011) also found increased anethole content in all organic and biological fertilizers treatments as compared to the control. In present study, *cis*-anethole was identified as a minor constituent varying from 0.77 to 1.73%. Limonene showed significant variation from 3.61 (in 26 t cow manure ha^{-1}) to 20.98% (in 104 t cow manure ha^{-1}). Mahmoud *et al.* (2016) reported higher limonene (40.0, 43.2 and 40.5%) content in treatments of a mixture of manure and compost, biofertilizer and mixture of manure, compost and biofertilizer, respectively, as compared to the control (38.5%).

Dill apiole content in treatments varied from 6.36 to 21.95% with highest contents in 26 t cow manure ha^{-1} (Table 5). Phytol content was highest (8.83%) in 26 t cow manure ha^{-1} amendment and lowest (0.25%) in 52 t cow manure ha^{-1} amendment. The minor constituents were also significantly affected by different amendments of cow manure and showed some significant variations such as terpinolene (0.82-3.89%), exo-fenchyl acetate (0.90-1.50%), methyl chavicol (1.25-1.95%) and neophytadiene (0.64-1.49%). Essential oil composition of aromatic plants depends on environmental and genetic factors that affect genetic expression (Bernath, 1986). The essential oil of fennel

Table 5: Essential oil composition of fennel grown in cow manure amended Karulanala soil

Compound name	RI		Percentage of oil				Mode of identi-fication
	Calcu-lated	Adams (2007)	0 t ha^{-1}	26 t ha^{-1}	52 t ha^{-1}	104 t ha^{-1}	
Myrcene	987	988	0.25	0.06	0.09	0.35	a,b
Decane	995	1000	0.48	0.07	0.37	0.06	a,b
Limonene	1028	1024	$14.14^a \pm 1.1$	$3.61^b \pm 0.5$	$5.32^b \pm 0.9$	$20.98^c \pm 1.0$	a,b,c
(*Z*)-*β*-Ocimene	1032	1032	1.51	0.52	0.65	2.06	a,b
Terpinolene	1089	1086	3.89	0.82	1.35	1.48	a,b
Undecane	1095	1100	0.46	0.07	0.28	0.06	a,b
Pentyl cyclohexa-1,3-diene	1156	1156	0.45	0.16	0.24	0.30	a,b
Methyl chavicol	1199	1195	1.25	1.16	1.55	1.95	a,b
exo-Fenchyl acetate	1233	1229	0.90	0.96	1.29	1.50	a,b
Cis-anethole	1252	1249	0.88	0.77	0.95	1.73	a,b
Trans-anethole	1287	1282	$39.87^a \pm 1.0$	$47.80^b \pm 0.2$	$56.05^c \pm 0.3$	$54.29^d \pm 0.7$	a,b,c
(*E*)-*β*-Farnesene	1454	1454	0.82	0.76	0.65	0.47	a,b
β-Sesquiphellandrene	1523	1521	4.32	3.98	3.37	1.27	a,b
Dill apiole	1628	1620	$18.53^a \pm 0.6$	$21.95^b \pm 0.2$	$18.84^a \pm 1.0$	$6.36^c \pm 0.2$	a,b
Ethyl hexyl benzoate	1698	-	0.24	0.32	0.24	0.18	a,b
Neophytadiene	1835	-	1.25	1.49	0.73	0.64	a,b
Phytone	1842	-	0.33	0.49	0.21	0.13	a,b
Phytol	1949	1942	$4.94^a \pm 0.1$	$8.83^b \pm 0.3$	$0.25^c \pm 0.1$	$1.97^c \pm 0.0$	a,b
Ethylene brassylate	2003	-	0.41	0.72	0.47	0.36	a,b
(*Z*)-Falcarinol	2034	2035	0.80	1.11	0.61	0.17	a,b
Total			95.72%	95.65%	93.51%	96.31%	
MH			19.79	5.01	7.41	24.87	
PP			60.53	71.68	77.39	64.33	
AH			0.94	0.14	0.65	0.12	
BCM			0.90	0.96	1.29	1.50	
SH			6.39	6.23	4.75	2.38	
OH			0.33	0.49	0.21	0.13	
ADTA			4.94	8.83	0.25	1.97	
ML			0.41	0.72	0.47	0.36	
Others			1.49	1.59	1.09	0.65	

a = Retention index (RI) relative to n-alkane homologous series (C_9-C_{33}) on Rtx-5 column; b = NIST, WILLEY library and MS data and literature (Adams, 2007); c = Co-injection with standards; SD = Standard deviation; RT = Retention time; MH: Monoterpene hydrocarbon (1, 3, 4, 5); BCM: Bicyclic monoterpenoids (9); SH: Sesquiterpene hydrocarbon (12, 13, 16); PP: Phenyl propanoids (8, 10, 11, 14); AH: Alkane hydrocarbon (2, 6); OH: Oxygenated hydrocarbon (17); ADTA: Acyclic diterpene alcohol (18); ML: Macrocyclic lactone (19); Other (7, 15, 20)

The mean values ($\pm$ standard deviation) superscripted with same alphabet in the same row do not significantly differ from each other at $p \leq 0.05$ level.

showed highest content of phenylpropanoids and lowest content of acyclic diterpene alcohol in 52 t ha^{-1} cow manure amendment. Monoterpene hydrocarbons content was highest and sesquiterpene hydrocarbon content was lowest in 104 t ha^{-1} cow manure amendment, respectively. Positive significant correlation was found between the Cu content in plant and sesquiterpene hydrocarbons ($r^2 = 0.969$, $P<0.05$) (Table 3). Some correlations were also observed between different classes of compounds of *C. sativum* by Punetha *et al.* (2018).

Conclusion: The results of this study revealed that the cow manure amendment to soil reduced Zn, Ni, Cu and Cr content in plant tissues. The application of 52 t cow manure ha^{-1} resulted in the highest plant growth. *Trans*-anethole also showed the highest percentage at 52 t ha^{-1} cow manure amendment and this was 40.58% more than the control as well as resulted in significant reduction in metal content.

Acknowledgement: This work was supported by University Grant Commission, (UGC), New Delhi, India [sanctioned vide grant No. 17-10/98(SA-1)]. The authors are grateful to UGC, New Delhi for the financial assistance and Head, Department of Chemistry for providing the necessary laboratory facilities.

Disclosure statement: No potential conflict of interest was reported by all the authors.

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