Evaluation of phytoaccumulation potential of toxic metals from sewage sludge by high-value aromatic plant geranium

A. Mazeed, N.B. Lothe, A. Kumar, S.K. Sharma, S. Srivastav and R.K. Verma*

Division of Agronomy and Soil Science, CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow-226 015, India

*Corresponding Author Email : rajesh.verma@cimap.res.in

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Abstract

Aim: The present study aimed to assess the phytoextraction potential of plant geranium (CIM BIO-171) to remove metals from sewage sludge and to investigate the growth, yield and quality of essential oil of this aromatic plant grown in contaminated sludge.

Methodology: The experiment was conducted at research farm of CSIR-CIMAP, Lucknow during 2017-18 in vertical cemented column pots dug into soil. Six treatment groups of sewage sludge and soil were taken with four replication, i.e., T₁-100% sewage sludge, T₂-80% sewage sludge + 20% soil, T₃-60% sewage sludge + 40% soil, T₄-40% sewage sludge + 60% soil, T₅-20% sewage sludge + 80% soil and T₆-soil (only soil). Experimental soil was analysed at pre-harvest and post-harvest stage for soil characters. All plant attributes were recorded at the time of harvesting.

Results: Result of the present study showed reduction in the heavy metals in the soil at harvest in comparison to the contents observed in soil at initial stage. All the parameters of growth, yield, biomass and essential oil were found to increase with the application rate of sewage sludge in geranium (CIM BIO-171).

Interpretation: The results of the present study clearly indicate the phytoextraction capability of geranium plant (CIM BIO-171). Furthermore, the plants grown in contaminated sludge has all the beneficial impact on growth, yield and quality of essential oil.

Key words: Geranium, Phytoextractor, Sewage sludge, Toxic metals

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Introduction

Sewage sludge obtained as a byproduct of sewage treatment plants are rich in heavy metals, soil nutrients (nitrogen and phosphorus), organic matter and trace elements like iron, copper, zinc, boron, molybdenum, and manganese. They are beneficial for plant growth as well as productivity (Abd-Alla *et al.*, 1999; Bhatt *et al.*, 2015). After the cleanup of heavy metals, sewage sludge can be used as a suitable substitute for commercial organic fertilizers for agricultural land *vis-à-vis* reduces the risk for soil and water contamination. The production of sewage sludge has increased due to rapid increase in urbanization and population. It is estimated that Indian cities and towns all together generate, approximately 19127 tons of sludge per day (Press Information Bureau: Achieving the Objective of Swachh and Swastha Bharat-Report, (NIC) Govt. of India, 2015). To manage these wastewater treatment plants effectively and efficiently, it is important to develop sustainable sewage sludge treatment technologies that cannot only reduce the toxicity of sludge but also prevent environmental pollution.

Land application is generally considered to be the most effective, economical and beneficial way of solid waste sludge disposal (Metcalf and Eddy, 2003), when compared with other methods such as incineration or land-filling (Sanchez-Monedero *et al.*, 2004). It is expensive as well as produce environmental hazards (Mazen *et al.*, 2010). Thus, recycling of solid waste sludge can be used for sustainable agricultural management as well as providing an appealing solution for nutrient cycling, which is an integral part of all natural processes. As sewage sludge is rich in organic matter and contain essential macro and microelements, it can be used for improving the physio-chemical (*i.e.*, physical, chemical, and biological) properties of soil which is essential for maintaining soil fertility and productivity (Arsalan *et al.*, 2007; Antonkiewicz and Pelka, 2014).

Moreover, a rich amount of organic matter helps in carbon sequestration and mitigation of greenhouse gas emissions (Jose *et al.*, 2010). Implementation of waste sewage sludge to soils as fertilizers in arid and semi-arid areas is beneficial as these soils have low amount of organic matter and other important nutrients. Mostly conventional chemical fertilizers do not provide organic carbon and some essential micronutrients, thus sewage sludge can be a better choice for plant growth and can be applied to less organic matter and micronutrient-deficient soils. Numerous researchers have reported an increased bioavailability of metals in sludge-amended soil (Golui *et al.*, 2014; Pandey *et al.*, 2015; Antonkiewicz and Pelka, 2014; Kulling *et al.*, 2001; Lopez-Mosquera *et al.*, 2000). Moreover, the primary nutrients present in sludge are organic which are not soluble as compared to chemical fertilizers and released slowly. Thus, solid waste sewage sludge can nourish plants at a slower rate over a longer period with higher efficiency, resulting in implementation of organic carbon in sludge-amended soil, thereby increasing several folds compared to inorganic fertilizer amended soils.

Previous studies have reported that geranium can be used both as a phytoextractor and for bioremediation against heavy metal contamination (Pandey *et al.*, 2015; Chand *et al.*, 2016). Geranium is an essential oil-bearing aromatic plant and commercially propagated by vegetative propagation or can be grown from seeds. Essential oils obtained from aromatic plants are free from the risk of heavy metals accumulation from plant biomass and these heavy metals do not enter the human food chain directly through phytoremediation by aromatic plants (Gupta *et al.*, 2013). Keeping in view the above, the aim of this study was to evaluate the growth, yield and phytoextraction potential of heavy metals by geranium plants. *vis-a-vis* assess the residual nutrients status.

Materials and Methods

Experimental area and design: The experiment was conducted in a research farm of CSIR-Central Institute for Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow, India for the duration of one year, *i.e.*, 2017–18. The experimental area experiences sub-tropical humid climatic condition. The experiment was conducted in a cylindrical RCC column pots dug vertically into the soil to avoid the exchange of pollutants and soil nutrients among the treatments. The RCC column pots measured 30 cm in diameter and 125 cm of high, embedded in the soil to a depth of 100 cm, keeping 25 cm above ground. The experiment was conducted in a completely randomized block design with four replications. A high yielding variety of geranium cv. CIM BIO-171 was selected as a test plant. Sewage sludge was collect from Bharwara Sewage Treatment Plant (STP), Gomti Nagar Lucknow, (India). It was air-dried and processed. [Treatments details are as follows: T₁ (100 % sewage sludge), T₂ (80 % sewage sludge + 20% soil), T₃ (60% sewage sludge + 40% soil), T₄ (40% sewage sludge + 60% soil), T₅ (20% sewage sludge + 80% soil) and T₆ (only soil, CIMAP research farm soil). Soil and sewage sludge were mixed properly in different ratios and filled according to their respective treatments. Filled RCC column pots with treatments were left for 20 days incubation period and regularly irrigation was done at different time intervals. After incubation, rooted cuttings of selected variety of geranium, which were healthy and uniformly growing in the nursery, were transplanted in the RCC column pots. All plant attributes were recorded at the time of harvest and the plant samples were collected for oil extraction and analyses of heavy metals and micronutrients.

Soil sampling and analyses: Soil samples were collected and processed with mortar-pestle and sieved with a 0.2 mm sieve for chemical analysis after 20 days incubation to analyse soil properties. Conductivity and pH meter (Consort C861 model) was used to measure the electrical conductivity of soil samples. Soil organic carbon was analyzed by Walkley and Black method (1934). Mineralizable nitrogen was determined by the method of Subbiah and Asija (1956). Exchangeable potassium was extracted with neutral normal ammonium acetate (NH₄OAC) and analyzed by flame photometry (Jackson, 1973). Available phosphorous was determined by the method of Olsen *et al.*

(1954). For heavy metals and micronutrients (Cr, Cd, Ni, Pb, Fe, Mn, Cu, Zn) analyses, collected samples were digested in an acid mixture (HF:HNO₃:HClO₄:H₂SO₄@3:3:2:1) (Baker and Amacher, 1982) on FOSS digester unite (Labtec™ Line) and digested materials were filtered through Whatman no. 42 filter paper and filtrates were diluted up to 50 ml with double distilled water in 50 ml volumetric flasks. The concentration were analyzed with inductively coupled plasma-optical emission spectrometry (ICP-OES), Perkin Elmer model 53000V.

Evaluation of growth and yield attributes: Plant growth attributes such as plant height, numbers of branch per plant, leaf: stem ratio, and shoot: root ratio recorded at plant growth stage and at harvest. The above surface biomass was harvested for the extraction of essential oil and yield estimation.

Heavy metal analyses in plants and factor calculation: Collected shoot (leaves and stems separately) and root sample of plants were cleaned with 0.1N HCl followed by deionized distilled water and then oven-dried at 70°C and acid digested (HNO₃:HClO₄ ¼ 10:4) for analyses of micronutrients and heavy metals (Piper, 1966; Jones and Case, 1990; Page *et al.*, 1982). The concentration of heavy metals and micronutrients was estimated with inductively coupled plasma-optical emission spectrometry (ICP-OES), Perkin Elmer model 53000V. Translocation factor (TF) and bio-accumulation factor (BAF) are used for identifying the test plant suitable for phytoremediation. TF and BAF of all four heavy metals were (Cr, Cd, Pb and Ni) calculated by the formula given by Chandra *et al.*, (2017).

Extraction and analysis of essential oil: Extraction of essential oil from fresh biomass was carried out by hydro-distillation in a Clevenger-type apparatus for 2 hrs (Clevenger, 1928). Distilled essential oil was dried over anhydrous Na₂SO₄ and stored at 4°C prior to analysis, followed by GC analysis of essential oils, carried out on a Perkin-Elmer Auto System XL gas chromatograph, equipped with a DB-5 capillary column (50m x 0.32 mm i.d., film

thickness 0.25 µm) and flame ionization detector. Identification of essential oil constituents was done based on retention time (RT) and retention index (RI). The relative amount of individual components was calculated based on the GC peak area (FID response).

Statistical analyses: Statistical analyses for various recorded observations were performed by SPSS 17 software package for Windows and subjected to ANOVA. Duncan's test was used to determine significance between the values.

Results and Discussion

The chemical analysis of sewage sludge collected from STP, Bharwara sewage treatment plant are as follows: soluble salts concentration (EC)-1627.6 µScm⁻¹; pH-7.7; carbon-10.3%; mineralizable nitrogen-611.8 mg kg⁻¹; phosphorus-69.1 mg kg⁻¹; potassium-205 mg kg⁻¹; total Fe- 11,611.4 mg kg⁻¹; Cu-614.7 mg kg⁻¹; Mn-2821.0 mg kg⁻¹; Zn-1426.5 mg kg⁻¹; Cr-425.0 mg kg⁻¹; Cd-20.9 mg kg⁻¹; Pb-181.2 mg kg⁻¹ and Ni-90.2 mg kg⁻¹ (Table 1, Fig. 1 and 2). The chemical properties of initial soil sample which was used in the experiment after 20 days of incubation, before planting are as follows: EC- 143.3 µScm⁻¹, pH-8.8, soil organic carbon -1.3%; mineralizable nitrogen-110 mg kg⁻¹; phosphorus-13.1 mg kg⁻¹; potassium-123.6 mg kg⁻¹; total Cr -34.4 mg kg⁻¹; Cd-3.8 mg kg⁻¹; Pb- 24.1 mg kg⁻¹; Ni -36.5 mg kg⁻¹; Fe - 4685.3 mg kg⁻¹; Cu -113.7 mg kg⁻¹; Mn- 2412.3 mg kg⁻¹ and Zn- 528.75 mg kg⁻¹ (Table 1, Fig. 1 and 2), respectively.

The perusal of data revealed that the application of sewage sludge significantly increased the soil reactions (pH, EC), macro (mineralizable N, P and K), micronutrients (Fe, Zn Cu and Mn) and heavy metals status (Table 1; Fig. 1 and 2), which might be due to the presence of significant amount of mineralizable nitrogen and degradable organic matter present in sewage sludge (Martinez *et al.*, 2002; Walter *et al.*, 2000). The pH of the treatments decreased with increase in the application ratio of

Table 1: Chemical composition of sewage sludge and soil at initial and post-harvest stage

Observation Treatment	EC (µS cm ⁻¹)		pH		SOC (%)		Available N (mg kg ⁻¹)		Available P (mg kg ⁻¹)		Available K (mg kg ⁻¹)	
Analysis	Initial stage	Post-harvest stage	Initial stage	Post-harvest stage	Initial stage	Post-harvest stage	Initial stage	Post-harvest stage	Initial stage	Post-harvest stage	Initial stage	Post-harvest stage
T ₁	1627.6 ^f	566.3 ^d	7.7 ^a	6.8 ^a	10.3 ^f	6.1 ^f	611.8 ^e	505.8 ^f	69.1 ^a	39.3 ^a	205 ^f	57.4 ^b
T ₂	1405.7 ^e	445.5 ^c	7.8 ^b	6.9 ^b	8.9 ^e	3.8 ^e	465.8 ^d	331.6 ^e	40.6 ^e	30.1 ^d	197.1 ^e	49.3 ^a
T ₃	1232.8 ^d	354.3 ^b	7.8 ^b	7.1 ^c	5.7 ^d	2.7 ^d	340.3 ^c	270.6 ^d	30.5 ^d	20.5 ^c	154.4 ^d	49.4 ^a
T ₄	822.1 ^c	303.5 ^b	7.9 ^c	7.2 ^c	3.9 ^c	1.6 ^c	329.7 ^c	243.7 ^c	24.4 ^c	15.7 ^b	148.7 ^c	43.2 ^a
T ₅	530.2 ^b	189.7 ^a	8.2 ^d	7.5 ^d	2.6 ^b	1.3 ^b	237.9 ^b	168.4 ^b	18.4 ^b	13.1 ^b	138.8 ^b	43.7 ^a
T ₆	147.3 ^a	143 ^a	8.8 ^e	8.2 ^e	1.3 ^a	0.5 ^a	110 ^a	55.2 ^a	13.1 ^a	7.5 ^a	123.6 ^a	47.8 ^a

Mean followed by same letter within one column (EC, pH, SOC, available N, available P and available K) do not differ significantly at $p \leq 0.05$ (Duncan's Multiple Range Test); *SOC- soil organic carbon; EC-electrical conductivity; N-nitrogen; P-phosphorus and K-potassium

Table 2 (a): Effect of different rates of sewage sludge on growth, herb yield and oil content

Observations Treatment	Height (cm)	Canopy (cm)		Biomass (kg pot ⁻¹)	Oil content (%)	Leaf: Stem ratio	Shoot: Root ratio
		NxS	ExW				
T ₁	63.0 ^c	102.5 ^d	103.7 ^{bc}	1.0 ^b	0.33 ^{ab}	2.1 ^{abc}	32.8 ^{ab}
T ₂	59.0 ^{bc}	90.0 ^{bc}	95.7 ^b	1.23 ^b	0.38 ^b	1.7 ^{ab}	41.4 ^{bcd}
T ₃	60.3 ^{bc}	92.5 ^{bc}	102.5 ^{bc}	1.07 ^b	0.29 ^a	2.2 ^{bc}	35.6 ^{abc}
T ₄	61.3 ^c	96.3 ^{cd}	108.7 ^c	1.25 ^b	0.33 ^{ab}	2.1 ^{abc}	45.9 ^{cd}
T ₅	48.7 ^{ab}	85.0 ^b	93.7 ^b	1.17 ^b	0.33 ^{ab}	2.4 ^c	50.0 ^d
T ₆	40.7 ^a	64.0 ^a	77.3 ^a	0.305 ^a	0.28 ^a	1.5 ^a	26.4 ^a

Mean followed by same letter within one column (height, canopy, biomass, L:S, S:R and oil content) do not differ significantly at $p \leq 0.05$ (Duncan's Multiple Range Test); *NxS-north to south, ExW-east to west

Table 2 (b): Effect of different rates of sewage sludge on dry matter production by different plant parts (root, stem and leaves)

Observation Treatment	Fresh weight of root (g pot ⁻¹)	Dry weight of root (g pot ⁻¹)	Fresh weight of leaves (g pot ⁻¹)	Dry weight of leaves (g pot ⁻¹)	Fresh weight of stem (g pot ⁻¹)	Dry weight of stem (g pot ⁻¹)
T ₁	31.8 ^b	8.2 ^b	672.8 ^b	116.1 ^b	326.4 ^b	38.8 ^{ab}
T ₂	29.9 ^b	7.5 ^b	762.1 ^b	135.2 ^b	463.2 ^b	55.1 ^b
T ₃	30.8 ^b	7.9 ^b	739.7 ^b	126.8 ^b	332.5 ^b	41.8 ^{ab}
T ₄	27.2 ^b	6.5 ^b	840.5 ^b	135.7 ^b	406.0 ^b	48.2 ^c
T ₅	24.0 ^b	5.9 ^b	814.2 ^b	137.3 ^b	356.1 ^b	44.8 ^b
T ₆	11.3 ^a	3.1 ^a	173.9 ^a	57.6 ^a	121.5 ^a	21.9 ^a

Mean followed by same letter within one column (fresh and dry matter produce by root, stem and leaves) do not differ significantly at $p \leq 0.05$ (Duncan's Multiple Range test)

Table 3 (a): Bio-concentration factor (BCF), Bio-accumulation factor (BAF) and Translocation factor (TF) of micronutrients in geranium at different rates of sewage sludge application

Observation Treatment	Fe			Cu			Mn			Zn		
	BCF	BAF	TF	BCF	BAF	TF	BCF	BAF	TF	BCF	BAF	TF
T ₁	0.29 ^a	0.12 ^a	0.41 ^c	0.09 ^a	0.07 ^a	0.74 ^a	0.06 ^a	0.13 ^a	2.12 ^c	0.19 ^a	0.35 ^a	1.79 ^b
T ₂	0.44 ^a	0.13 ^a	0.29 ^b	0.22 ^a	0.20 ^{ab}	0.97 ^a	0.08 ^a	0.15 ^a	2.10 ^c	0.40 ^b	0.65 ^b	1.64 ^b
T ₃	0.75 ^a	0.23 ^{ab}	0.31 ^b	0.37 ^b	0.26 ^b	0.73 ^a	0.20 ^b	0.39 ^b	1.93 ^c	0.65 ^c	0.88 ^c	1.38 ^a
T ₄	2.42 ^c	0.31 ^b	0.13 ^a	0.80 ^c	0.51 ^c	0.65 ^a	0.41 ^e	0.45 ^c	1.11 ^a	0.91 ^d	1.14 ^d	1.25 ^a
T ₅	1.64 ^b	0.49 ^c	0.30 ^b	0.05 ^a	0.06 ^a	1.42 ^b	0.26 ^c	0.62 ^e	2.41 ^d	0.16 ^a	0.28 ^a	1.77 ^b
T ₆	1.42 ^b	0.91 ^d	0.67 ^d	0.14 ^a	0.19 ^{ab}	1.39 ^b	0.31 ^d	0.51 ^d	1.66 ^b	0.67 ^c	1.06 ^c	1.59 ^b

Mean followed by same letter within one column (BCF, BAF and TF) do not differ significantly at $p \leq 0.05$ (Duncan's Multiple Range Test); *Fe-iron, Cu-copper, Mn-manganese, Zn-zinc; *TF- translocation factor, BCF- bio-concentration factor and BAF- bio-accumulation factor

sewage sludge. The heavy metals content was present in toxic level in all the treatments (all proportions of sewage sludge), except for T₆ (only soil) (higher as compared to permissible limits to agriculture soils), which may be attributed to the fact that the chemical composition of added sludge directly affects the soil chemical properties (Barzegar *et al.*, 2002; Veeresh *et al.*, 2003). The amendment of sewage sludge reduces soil pH and increases total soluble salts of the soil (Antoline *et al.*, 2005). In another study, Stamatiadis *et al.* (1999) reported that decomposition of

sludge makes soil humic causing nitrification of N-NH_4^+ and reduction of soil pH. A significant variation was observed in plant height, canopy and leaf: stem ratio in all the treatments (Table 2a). Plant height in all sewage sludge applied treatments displayed significant variation than control (T₆). The plant height was maximum in T₁ (63.0 cm), followed by T₄ (61.3 cm) and T₃ (60.3 cm). The plant canopy, leaf: stem and shoot: root ratio also varied significantly among the treatments. The maximum plant canopy was recorded in treatment T₁ (102.5 cm NxS and 103.7 cm ExW).

Table 3(b): Bio-concentration factor (BCF), Bio-accumulation factor (BAF) and Translocation factor (TF) of heavy metals in geranium at different rates of sewage sludge application

Observation Treatment	Cr			Cd			Pb			Ni		
	BCF	BAF	TF	BCF	BAF	TF	BCF	BAF	TF	BCF	BAF	TF
T ₁	0.02 ^a	0.02 ^a	1.68 ^{ab}	0.26 ^a	0.33 ^a	1.24 ^c	0.05 ^a	0.10 ^a	2.03 ^a	0.06 ^a	0.09 ^a	1.54 ^a
T ₂	0.02 ^a	0.04 ^a	1.74 ^{ab}	0.38 ^{ab}	0.28 ^a	0.74 ^{ab}	0.06 ^a	0.13 ^a	2.75 ^{ab}	0.12 ^a	0.19 ^{ab}	1.72 ^{ab}
T ₃	0.03 ^a	0.03 ^a	1.01 ^{ab}	0.66 ^{ab}	0.37 ^a	0.58 ^a	0.12 ^a	0.16 ^a	1.45 ^a	0.14 ^a	0.26 ^b	2.14 ^{ab}
T ₄	0.05 ^a	0.03 ^a	0.76 ^a	0.79 ^b	0.37 ^a	0.57 ^a	0.20 ^b	0.30 ^b	1.52 ^a	0.29 ^b	0.44 ^c	1.54 ^a
T ₅	0.03 ^a	0.07 ^b	3.09 ^c	0.70 ^b	0.64 ^b	1.01 ^{bc}	0.10 ^a	0.36 ^b	4.72 ^b	0.32 ^b	0.64 ^d	2.07 ^{ab}
T ₆	0.22 ^b	0.33 ^c	2.08 ^b	1.25 ^c	0.74 ^b	0.66 ^{ab}	0.22 ^b	1.06 ^c	5.19 ^b	0.60 ^c	1.37 ^e	2.27 ^b

Mean followed by same letter within one column (BCF, BAF, and TF) do not differ significantly at $p \leq 0.05$ (Duncan's Multiple Range Test); *Cr-Chromium; Cd-cadmium; Pb-lead; Ni-nickel; *TF- translocation factor; BCF- bio-concentration factor and BAF- bio-accumulation factor

Table 4: Effect of different rates of sewage sludge on essential oil quality of geranium

Chemical constituents	Compound R.T.	Content (%)					
		T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
α-Pinene	9.48	0.1	0.5	0.3	0.5	0.4	0.1
Myrcene	11.7	0.1	0.1	0.1	0.1	0.1	t
α-Phellandrene	-	-	-	-	-	-	-
p-Cymene	12.94	t	0.1	0.1	0.1	0.1	t
Limonene	13.12	0.2	0.2	t	0.2	0.2	0.1
(Z)-β-Ocimene	-	-	-	-	-	-	-
(E)-β-Ocimene	-	-	-	-	-	-	-
Linalool	16.14	2.0	2.2	2.6	2.0	3.4	1.4
Cis-Rose oxide	16.69	0.2	0.3	0.3	0.4	0.3	0.3
trans-Rose oxide	17.48	0.1	0.2	0.2	0.2	0.2	0.2
Isomenthol	19.31	6.5	7.3	8.1	7.6	7.0	5.4
α-Terpineol	20.16	0.1	0.1	0.1	0.1	0.1	0.1
Citronellol	22.16	24.8	24.9	28.2	28.7	25.5	26.6
Neral	22.90	0.8	0.9	0.9	0.8	0.8	0.6
Geraniol	23.24	24.8	23.6	20.5	18.6	25.5	19.6
Geranial	24.24	1.5	-	-	-	-	-
Citronellylformate	24.48	7.9	9.8	10.5	10.0	8.8	7.9
Geranylformate	25.72	4.4	4.8	4.1	4.0	3.9	3.6
Citronellyl acetate	28.00	0.3	0.6	0.6	0.5	0.5	0.5
Geranylacetate	29.36	0.5	0.6	0.7	0.7	0.5	0.6
β-Bourbonene	29.84	0.5	0.6	0.5	0.6	0.6	0.8
(E)-Caryophyllene	31.40	0.8	0.6	0.4	0.6	0.5	0.8
Citronellyl propionate	32.37	0.1	0.2	0.3	0.2	0.2	0.2
α-Humulene	32.97	0.3	0.2	0.1	0.2	0.2	0.3
Geranyl propionate	33.75	0.1	0.1	0.1	0.1	0.1	0.1
Germacrene D	34.14	1.7	1.2	0.5	1.2	1.2	1.8
2-Phenyl ethyl tiglate	38.33	1.3	1.1	1.1	1.2	0.9	1.5
10-epi-γ-eudesmol	40.07	8.1	6.1	6.2	6.4	6.1	8.2
Geranyltiglate	42.87	2.6	1.9	1.6	1.7	1.7	2.6

*RT- retention time

The maximum leaf: stem and shoot: root ratio was 2.4 and 50, respectively in T₅ treatment. Fresh biomass yield (shoot and root) significantly varied among all the treatments (Table 2a). The maximum fresh herb yield (biomass) was obtained in treatment T₄

(1.25 kg barrel⁻¹), followed by T₂ (1.23 kg barrel⁻¹) and minimum in T₆ (control) (0.305.5 kg barrel⁻¹). The order of percent increase in fresh herb yield with respect to control was in the following order T₄:76%> T₂:75%> T₅:74%> T₃:72%> T₁:70%. Overall, the results

Fig. 1: Effect of different rates of sewage sludge on total micronutrient concentration in soil at initial and post-harvest stage; Duncan's Multiple Range Test, significant at $p \leq 0.05$.

Fig. 2: Effect of different rates of sewage sludge on total heavy metal concentration in soil at initial and post-harvest stage; Duncan's Multiple Range Test, significant at $p \leq 0.05$.

revealed that geranium plant growth and yield attributes improved with sewage sludge application. Application of sludge to the soil increases the growth and yield potential by supplying additional nutrients as it contains high levels of organic matter, macro and micronutrients (Pandey *et al.*, 2019; Patel and Patra *et al.*, 2014a).

The percent oil content in all the treatments significantly varied with control (T_0) and ranged from 0.28% to 0.33%. It was found to be higher (26.32%) in T_2 treatment as compared to control (T_0) (Table 2a). In all the treatments, a good and market acceptable quality of essential oil was obtained. In Table, 29 chemical constituents were detected by Gas Chromatography.

The major chemical constituents in the treatments found to be in the following range: geraniol 18.6-25.5%, citronellol 24.8-28.7%, citronellyl formate 7.9-10.5%, isomenthol 5.4-8.1%, linalool 1.4-3.4% (Table 4). The essential oil quality in the all treatments was acceptable by aroma industry. Geranium essential oil chemical composition depends on soil fertility and climatic conditions of the growing areas (Patra *et al.*, 2000 and Pandey *et al.*, 2019).

Post harvest soil of all major soil chemical properties significantly affected in all treatment combinations (Table 1; Fig. 1, 2). Electrical conductivity, pH, organic carbon, available nitrogen, phosphorus and potassium, including heavy metals (Cr,

Fig. 3: Effect of different levels of sewage sludge on micronutrient and heavy metal (nickel, cadmium, and chromium) uptake in root, stem, and leaf of geranium; Duncan's Multiple Range Test, significant at $p \leq 0.05$.

Cd, Pb, and Ni) and micronutrients (Fe, Cu, Mn, and Zn) were reduced as compared to the initial status of the treatments. Reduction in electrical conductivity and soil pH may be due to leaching of soluble salts as well as uptake by the plants and mineralization of organic matter. The percent reduction in micronutrient content from initial (after 20 days of sewage sludge application and before planting) status ranged between iron-33%-76.4%, copper-31.8%-90.5%, manganese-72.81%-91.9% and zinc-18.3%-74% whereas chromium, cadmium, lead and nickel content reduced from 6.6%-49.1%, 40.2%-78.9%, 20.5%-67.6%, and 19.3%-76.4% compared to the initial status.

The chromium, cadmium, lead and nickel concentration in post-harvest soil decreased with respect to the concentration in soil prior planting (Pandey et al., 2015, 2019; Patel and Patra et al., 2014a; Glaser et al., 2002). Overall, it was observed that the application of sewage sludge increased the yield potential of crop by supplying plant nutrients as it has high levels of organic matter as well as other soil nutrients. Heavy metal concentration increased with an increasing proportion of sewage sludge added to the soil. The presence of high levels of organic carbon content represented high ionic strength in sewage sludge with higher soluble and exchangeable metals (Yoo et al., 2002).

The availability of heavy metals in soils depends on soil reaction and organic matter content (Karaca, 2004). Except for Cu uptake in leaf other micronutrients (Fe, Zn, and Mn) uptake in various plant parts significantly varied within the treatments. Plant roots showed higher accumulation of all micronutrients (Fe, Cu, Zn, and Mn) followed by leaf and stem. Fe uptake in different plants portion significantly varied among the treatments and maximum uptake was observed in treatment T₄ (5013 mg kg⁻¹), followed by T₅ (2648 mg kg⁻¹) in roots. Overall, it was observed that the order of micronutrient uptake was Fe > Zn > Mn > Cu in all plant parts (Fig. 1). Several researchers have reported that concentrations and accumulation of micronutrients in plant parts depend on their availability, in their respective treatments (Pandey et al., 2019; Chand et al., 2016).

The uptake of toxic metals such as Cr, Cd, Pb and Ni by all plant parts of the geranium present in sewage sludge was higher than the control (only soil) (Fig. 3e, f, g, h). The maximum accumulation of all the metals was recorded in the root portion followed by leaf and stem. The order of heavy metal uptake in sludge applied treatments were in the following order: Pb > Cr > Ni > Cd, Cr > Pb > Ni > Cd, Cr > Pb > Ni > Cd, Pb > Cr > Ni > Cd and Cr > Ni > Pb > Cd, respectively in T₁, T₂, T₃, T₄, and T₅ treatments. The accumulation trend of various heavy metals depended on the application of sewage sludge and soil ratio, pH, organic carbon content, chemical and biological status of the treatments. Overall, it was observed that all heavy metals in sewage sludge were present at a toxic level decreased and were converted into non-toxic form (level of the toxic metals within permissible limit); hence it may be used as bulky organic manures in agricultural lands. Translocation factor is the ratio of any metals concentration in the shoot with respect to concentration in the

root. This factor is an indicator of metal content, which gets transferred from root to shoot portion of a plant. Translocation factor (TF) and bio-concentration factor (BAF) are important for identifying the phytoremedial potential of plants (Mendez and Mair, 2007). Factors responsible for identifying phytoextraction potential, showed significant variation among the treatments (Table 3a, b). TF of Mn was >1 (ranged from 1.11 to 2.41) and Zn (ranged from 1.25 to 1.79) in all the treatments. Ni and Pb showed TF > 1 in all the treatments, while Cr had TF > 1 in most of the treatments (T₁, T₂, T₃, T₅, and T₆), except for T₄, and Cd TF >1 was in treatments T₁ and T₅. Higher accumulation of heavy metals in roots as compared to shoots may be due to complexation of heavy metals with some organic acids and sulphhydryl groups to restrict the translocation of heavy metals into shoot part. Root exudates stabilize, demobilize and bind heavy metals in rhizosphere soil, thereby reducing their mobility/bio-availability. Some aromatic plants (*Pelargonium graveolens* and *Cymbopogon citratus*), rice and tomato have used to immobilize metals in the soil through absorption and accumulation by roots (Singh et al., 2004; Singh and Agrawal, 2010; Gautam et al. 2017; Pandey et al., 2019).

BCF is the ratio of the concentration of metals in roots to a concentration of metals in soil whereas BAF is the ratio of metals concentration in the shoot to metals concentration in soil. In this study, BCF and BAF of various metals showed a significant difference among the treatments and most of the treatments had TF values >1. (Table 3a, b). Overall, it was observed that geranium accumulated high concentration of heavy metals from sewage sludge. Further, geranium, when used as a bioremediator, can phytostabilize as well as phytoextract some metals. It can be concluded that high-value aromatic crop geranium (CIM BIO-171) can be a suitable candidate for removal of heavy metals from sewage sludge. Moreover, the decontaminated sewage sludge can be used for improving the physical and chemical properties of the soil.

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References

- Abd-Alla, M.H., Yan-Feng and S. Schubert: Effect of sewage sludge application on Nodulation, nitrogen fixation, and plant growth of faba bean, soybean and Lupin. *Angewandte-Botanik*, Germany, **3/4**, 69-75 (1999).
- Antolín, M.C., I. Pascual, C. García, A. Polo and M. Sánchez-Díaz: Growth, yield and solute content of barley in soils treated with sewage sludge under semiarid Mediterranean conditions. *Field Crops Res.*, **2-3**, 224-237 (2005).
- Antonkiewicz, J. and R. Pelka: Fractions of heavy metals in soil after the application of municipal sewage sludge, peat, and furnace ash. *Soil Sci. Annu.*, **65**, 118-125 (2014).

- Arslan, A., M. M. Alzoubi, H. Nasralla, N. Bijon, J. Abdul Gawad and O. Jusadan: The effect of mixing sludge with surface soil layer on the physical properties and cotton yield. *Proceedings of the Workshop on sustainable management of wastewater for agricultural production in water scarce countries*. ICARDA, Aleppo, Syria, pp. 33-40 (2007).
- Baker, D. E. and M. C. Amacher: Nickel, copper, zinc and cadmium 1. *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Methods of Soil Analysis*, 2, 323-336 (1982).
- Barzegar, A.R., A. Yousefi and A. Daryashenas: The effect of addition of different amounts and types of organic materials on soil physical properties and yield of wheat. *Plant and Soil*, 2, 295-301 (2002).
- Bhatt, P., N. Mathur, A. Singh and P. Bhatnagar: Recycled sewage sludge: A step to sustainable agriculture. *Int. J. Chem. Sci.*, 4, 1611-1620 (2015).
- Chand, S., G. Singh, Rajkumari and D.D. Patra: Performance of rose scented geranium (*Pelargonium graveolens*) in heavy metal polluted soil vis-à-vis phytoaccumulation of metals. *Int. J. Phyto.*, 8, 754-760 (2016).
- Chandra, R., N. K. Dubey, V. Kumar and R. Kumar: *Phytoremediation of Environmental Pollutants*. 1st Edn., CRC Press, Taylor & Francis, Boca Raton (2017).
- Clevenger J.E.: Apparatus for determination of volatile oil. *J. Am. Pharma. Assoc.*, 17, 345-349 (1928).
- Gautam M., D. Pandey and M. Agrawal: Phytoremediation of metals using lemongrass (*Cymbopogon citratus* (DC) Stapf.) grown under different levels of red mud in soil amended with biowastes. *Int. J. Phytoreme.*, 6, 555-562 (2017).
- Glaser, B., J. Lehmann and W. Zech: Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal—a review. *Biol. Fertil. Soils*, 4, 219-230 (2002).
- Golui, D., S.P. Datta, R.K. Rattan, B.S. Dwivedi and M. C. Meena: Predicting bioavailability of metals from sludge-amended soils. *Environ. Monit. Assess.*, 12, 8541-8553 (2014).
- Gupta, A.K., S.K. Verma, K. Khan and R.K. Verma: Phytoremediation using aromatic plants: A sustainable approach for remediation of heavy metals polluted sites. *Environ. Sci. Technol.*, 18, 10115-10116 (2013).
- Jackson, M.L.: *Soil Chemical Analysis*. Prentice Hall of India (1973).
- Jones, J.B. and V.W. Case: Sampling, handling and analyzing plant tissue samples. In: *Soil Testing and Plant Analysis* (Ed.: R.L. Westerman). 3rd Edn., Madison, pp. 404-409 (1990).
- Jose, M., S. Disla, J. Navarro-Pedreño and I.G. Lucas: Contribution of a sewage sludge application to the short-term carbon sequestration across a wide range of agricultural soils. *Environ. Earth Sci.*, 8, 1613-1619 (2010).
- Karaca, A.: Effect of organic wastes on the extractability of cadmium, copper, nickel, and zinc in soil. *Geoderma*, 2-4, 297-303 (2004).
- Kulling, D., F. Stadelmann and U. Herter: Sewage sludge- fertilizer or waste? UKWIR Conference, Brussels (2001).
- Lopez-Mosquera, M.E., C. Moiron and E. Carral: Use of dairy industry sludge as fertilizer for grassland in northwest Spain: Heavy metal levels in the soil and plants. *Resour. Conser. Recy.*, 30, 95-109 (2000).
- Martinez, F., C. Cuevas, W. Teresa and I. Iglesias: Urban organic wastes effects on soil chemical properties in degraded semiarid ecosystem. In: *Seventeenth WCSS, Symposium*, 20, 1-9 (2002).
- Mazen, A., F.A. Faheed and A.F. Ahmed: Study of potential impacts of using sewage sludge in the amendment of desert reclaimed soil on wheat and jews mallow plants. *Brazilian Arch. Biol. Technol.*, 4, 917-930 (2010).
- Mendez, M.O. and R.M. Maier: Phytostabilization of mine tailings in arid and semiarid environments—an emerging remediation technology. *Environ. Hlth. Perspect.*, 3, 278-283 (2007).
- Metcalf and Eddy: *Wastewater engineering: Treatment, disposal and reuse*. 4th Edn., McGraw-Hill Publishing Company Ltd., New York (2003).
- Olsen, S.R.: Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Washington, United States Department of Agriculture (1954).
- Page, A.L., R.H. Miller and D.R. Keeney: *Methods of Soil Analysis. Part 2. American Society of Agronomy. Soil Science Society of America*, Madison, WI, USA (1982).
- Pandey, J., S. Chand, S. Chaurasiya, R. Kumari, D.D. Patra, R.K. Verma and S. Singh: Effect of tannery sludge amendments on the activity of soil enzymes and phytoremediation potential of two economically important cultivars of geranium (*Pelargonium graveolens*). *Soil Sedi. Contami.*, 4, 395-410 (2019).
- Pandey, J., S. Chand, S. Pandey and D.D. Patra: Palmarosa [*Cymbopogon martinii* (Roxb.) Wats.] as a putative crop for phytoremediation, in tannery sludge polluted soil. *Ecotoxicol. Environ. Saf.*, 122, 296-302 (2015).
- Patel, A. and D.D. Patra: Influence of heavy metal rich tannery sludge on soil enzymes vis-à-vis growth of *Tagetes minuta*, an essential oil bearing crop. *Chemosphere*, 112, 323-332 (2014a).
- Patra, D.D., M. Anwar and S. Chand: Integrated nutrient management and waste recycling for restoring soil fertility and productivity in Japanese mint and mustard sequence in Uttar Pradesh, India. *Agricul. Ecosyst. Environ.*, 3, 267-275 (2000).
- Piper, C.S.: *Soil and plant analysis*. NY: Interscience Publisher (1966).
- Press Information Bureau, Government of India, Achieving the Objective of Swachh and Swastha Bharat-Report, National Informatics Centre (NIC), New Delhi (2015).
- Sanchez-Monedero, M.A., C. Mondini, M. De Nobili, L. Leita and A. Roig: Land application of biosolids. Soil response to different stabilization degree of the treated organic matter. *Waste Manage.*, 4, 325-332 (2004).
- Singh, R.P. and M. Agrawal: Variations in heavy metal accumulation, growth and yield of rice plants grown at different sewage sludge amendment rates. *Ecotoxicol. Environ. Saf.*, 4, 632-641 (2010).
- Singh, S., S. Sinha, R. Saxena, K. Pandey and K. Bhatt: Translocation of metals and its effects in the tomato plants grown on various amendments of tannery waste: Evidence for involvement of antioxidants. *Chemosphere*, 57, 91-99 (2004).
- Stamatiadis, S., J.W. Doran and T. Kettler: Field and laboratory evaluation of soil quality changes resulting from injection of liquid sewage sludge. *Appl. Soil Ecol.*, 3, 263-272 (1999).
- Subbiah, B.V. and A.L. Asija: A rapid method for determination of available nitrogen in soil. *Curr Sci.*, 25, 259-260 (1956).
- Veeresh, H., S. Tripathy, D. Chaudhuri, B. Ghosh, B. Hart and M. Powell: Changes in physical and chemical properties of three soil types in India as a result of amendment with fly ash and sewage sludge. *Environ. Geol.*, 5, 513-520 (2003).
- Walkley, A. and I.A. Black: An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.*, 1, 29-38 (1934).
- Walter, I., G. Cuevas, S. Garcia and F. Martinez: Biosolid effects on soil and native plant production in a degraded semiarid ecosystem in central Spain. *Waste Manag. Res.*, 3, 259-263. (2000).
- Yoo, M.S. and B.R. James: Zinc extractability as a function of pH in organic waste-amended soils. *Soil Sci.*, 167, 246-259 (2002).