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Influence of metals on essential oil content and composition of lemongrass (*Cymbopogon citratus* (D.C.) Stapf.) grown under different levels of red mud in sewage sludge amended soil

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HIGHLIGHTS

- Metal contents increased in leaves of lemongrass grown in red mud amended soil.
- Red mud treatments in soil increased the essential oil content in lemongrass.
- Major constituents of essential oil also modified under different red mud treatments.
- Citral content was maximum under 10% (w/w) red mud treatment.
- Metal contents in essential oil were within FSSAI limits for food.

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ABSTRACT

Lemongrass is a commercially important perennial herb with medicinal value and ability to tolerate high alkaline and saline conditions. Essential oil bearing plants can grow safely in soil contaminated with heavy metals without severe effects on morphology and oil yield. The present study was aimed to assess the essential oil content and composition in lemongrass in response to elevated metals in above-ground plant parts. Pot experiment was conducted for six months using sewage sludge as soil amendment (soil: sludge: 2:1 w/w) followed by red mud treatments (0, 5, 10 and 15% w/w). Garden soil without sludge and red mud was control and there were ten replicates of each treatment. Oil content in leaves was differently affected due to presence of metals in soil under different treatments. Oil content under S_{RM5} (5% red mud) treatment was raised by 42.9 and 11.5% compared to the control and S_{RM0} treatment, respectively. Among identified compounds in oil under red mud treatments, 17 compounds contributed more than 90% of total volatiles (citral contributing approximately 70%). Under S_{RM10} treatment, essential oil showed maximum citral content (75.3%). Contents of Fe, Zn, Cu, Cd, Ni and Pb in above-ground plant parts exceeded, whereas Mn was detected within WHO permissible limits for medicinal plants. However, metal contents in essential oil were well within FSSAI limits for food. The study suggests utilization of 5 and 10% red mud in sludge amended soil for lemongrass cultivation to have better oil yield and quality, without metal contamination.

1. Introduction

Increase in demand of aluminum for industrial, commercial and domestic purposes has led to an increase in generation of red mud. During the Bayer's process, per ton of alumina production generates 4–5 tons of red mud (Power et al., 2011), which is a highly alkaline and saline residue, mainly composed of iron(III) oxide (Fe_2O_3), aluminum oxide (Al_2O_3), calcium oxide (CaO), sodium oxide (Na_2O), titanium dioxide (TiO_2), silicon dioxide (SiO_2), phosphorus pentoxide (P_2O_5) and vanadium(IV) oxide (VO_5) (Gautam et al., 2016). India alone contributes nearly 6.25% of global annual red mud generation (Parlikar et al., 2011) and disposal of such a huge quantity of waste as a landfill may pose an environmental risk. Although, in recent years, there are several applications of red mud such as in making ceramics, building materials, pigments, paints, as an effective adsorbent and catalyst (Wang

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et al., 2008), however, reclamation and its utilization as a soil amendment can be cost effective and a sustainable approach towards management of this waste (Mahar et al., 2015; Xue et al., 2016).

High alkalinity, salinity, poor water holding capacity, presence of potentially toxic metals such as cadmium, nickel, chromium and lead and less availability of essential nutrients to plants are major constraints in using this waste for raising plants. It has been, however, shown that organic amendments (sewage sludge, animal manure, vegetative dry dust) counterbalanced the adverse effects of red mud (Xenidis et al., 2005; Chauhan and Silori, 2011). Gray et al. (2006) also showed that application of lower doses of red mud with organic amendments in soil is effective approach for the betterment of plant growth and yield, however, adverse effects are observed at higher dose. Edible crops grown in metal contaminated soil are often marked by reduced growth and yield due elevated levels of potentially toxic metals with high risk of food chain contamination (Balkhair and Ashraf, 2016). On contrary to this, quantity as well as quality of essential oil extracted from medicinal plants is less affected or unaffected by metal contaminants (Zheljazkov et al., 2006).

India is the second largest producer of essential oil after Brazil contributing about 25.80% of its annual production (Lawrence, 2009). *Cymbopogon citratus* commonly known as lemongrass belongs to family Poaceae and is widely cultivated in India due to its aromatic and medicinal properties. In Brazil, tea from lemongrass leaves is popularly used as spasmolytic, analgesic, antipyretic, diuretic, anti-inflammatory and tranquilizer (Ferreira, 1984). Essential oil content in leaves of lemongrass ranged between 1 and 2% on dry weight basis and is widely used in perfumery and cosmetics due to its high citral content with a strong lemony aroma (Carlini et al., 1986). Also, citral is a raw material for the production of ionone, vitamin A and β-carotene and therefore the oil is in high demand both in domestic as well as in international markets.

Israila et al. (2015) reported that lemongrass is a metal (lead, cadmium, zinc, chromium and cobalt) tolerant plant and can be cultivated for reclamation of heavy metal contaminated sites. Prasad et al. (2010) reported that although some medicinal plants are metal tolerant, but accumulation of metals in them somehow affects their essential oil yield and quality. Zheljazkov et al. (2008) evaluated the effects of metal enriched soils on selected medicinal plants and found that essential oil content in *Melissa officinalis* was maximum when grown in soil collected from 0.50 km followed by 3, 6 and 9 km from lead-zinc smelter near Plovdiv, Bulgaria, whereas essential oil content in *Marrubium vulgare* and *Origanum heracleoticum* increased with increase in distance from the smelter. Kumar and Patra (2012) reported minimum essential oil content (0.11%) in *Mentha piperita* when grown in 100% fly ash compared to the control. Patel et al. (2015) also found a significant reduction in essential oil yield and its constituents (linalool and methyl chavicol) in *Ocimum basilicum* cultivated in sewage sludge amended soil.

The present study was performed to evaluate the influence of varying red mud concentrations in soil amended with sewage sludge on content and chemical composition of essential oil extracted from lemongrass. The study would suggest some future prospects for utilization of red mud for cultivation of this medicinally and commercially important tropical grass.

2. Materials and methods

2.1. Study area

The experiment was carried out at the Botanical Garden of Banaras Hindu University (B.H.U.), Institute of Science, situated in the Eastern Gangetic Plains of India at 25°18′ N latitude, 82° 01′ E longitude and 76.19 m above sea level. The experiment was conducted during February to August 2013 when mean monthly maximum temperature ranged between 25.8 and 41.4 °C, mean monthly minimum temperature between 12.5 and 27.3 °C and total rainfall was 110.0 mm.

2.2. Experimental design and raising of plants

The pot experiment was conducted using garden soil, sewage sludge and red mud. Red mud in the form of dried lumps was collected from red mud dumping site of Hindustan Aluminum Corporation (HINDALCO), Renukoot, Sonbhadra. The test plant, lemongrass was procured from Department of Ayurveda, Institute of Medical Sciences, B.H.U. Dried sewage sludge cakes were collected from the sludge bed of Dinapur sewage treatment plant and garden soil from Botanical Garden, B.H.U. by digging out up to a depth of 30 cm. Visible stone particles and plant materials were removed from garden soil by sieving. Red mud, sewage sludge and garden soil were air dried, grinded uniformly to get a homogenous mass and passed through a 2 mm sieve. Soil was amended and mixed uniformly with sludge in the ratio of 2:1 (w/w). To sludge amended soil thus obtained, red mud was added to achieve S_{RM0}: 0%, S_{RM5}: 5%, S_{RM10}: 10% and S_{RM15}: 15% (w/w) concentrations. The pot without sludge amendment and red mud treatment in soil served as a control.

Different soil combinations were filled into cylindrical plastic pots (diameter 25 cm and height 50 cm) and there were ten replicates of each combination, totaling 50 experimental units. Each pot was filled with 10 kg soil-mixtures of respective treatments and left in garden for two weeks for stabilization. After maintaining same moisture level in each pot, one plant slip (15 cm shoot length and 5 cm root length) of lemongrass per pot was transplanted on fifteenth day of soil-mixture stabilization. Equal amount of tap water was used to water the pots every alternate day. Leakage of water from the pots was completely avoided.

2.3. Soil sampling and analysis

On fifteenth day of soil stabilization, soil samples from different treatments were collected in triplicate using a soil corer (5 cm diameter and 10 cm depth). Each of the collected sample was air dried and ground to pass through a sieve of 2 mm mesh size. Soil samples from different treatments along with red mud, sewage sludge and garden soil were analyzed for selected physico-chemical properties. The pH and electrical conductivity were measured using pH meter (Model EA940, Orion, U.S.A) and conductivity meter (Model 303, Systronics, India), respectively. Total organic carbon (TOC) and available phosphorous (P) were examined by the method described by Allison (1973) and Olsen et al. (1954), respectively whereas total nitrogen (TN) was analyzed using Gerhardt automatic N analyzer (Model KB8S, Germany). Exchangeable sodium percentage (ESP) through exchangeable cations (sodium, potassium, calcium and magnesium) was determined following repeated leaching method (Hesse, 1971) and the contents of exchangeable cations in soil were assessed using Atomic Absorption Spectrometry (Analyst-800, Perkin Elmer Inc., Norwalk, CT, USA). For metal analysis, soil samples from different treatments were oven dried at 80 °C till the constant weight was attained.

2.4. Isolation of essential oils

Fresh leaves (300 g) collected from control and different treatments at 180 days after transplantation (DAT), were thoroughly washed with distilled water and subjected to hydrodistillation in Clevenger type apparatus (Clevenger, 1928) for 3 h. Extraction of oil