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Evaluating the Effect of Sewage Sludge on Bioactive Components of *Momordicacharantia*

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Summary: *Momordicacharantia* L. belongs to the family Cucurbitaceae. This plant has widely been reported for use in traditional medical systems for the treatment of diabetes, rheumatism, intestinal gas and malaria. The ethnicity of *M. charantia*is Asia, Africa and some parts of Europe where it is considered edible. Sewage sludge, a by-product of waste water treatment plant, contains several nutrients. These nutrients are known as beneficial for the better growth and good yield of the plants. The objective of this study is to optimize soil with different ratios of sewage sludge for increased growth of bioactive components in *M. charantia*. The plant was grown at six places; in soil containing 20%, 40%, 60% and 80% sewage sludge ratio as well as in pure soil and in pure sewage sludge. Fruit harvesting was carried out after 75 days. Methanol extract of *M. charantia* (Fruit) was subjected to analysis using GC-MS. Comparative quantification of 7,11-hexadecadienal, oleic Acid and stigmasterol were carried out. It has been found that the soil containing 60% sewage sludge yielded maximum quantity of 7,11-hexadecadienal, oleic Acid and stigmasterol. Therefore, optimization of soil with different ratios of sewage sludge is carried out and 60% ratio of sewage sludge in soil is recommended for the promising growth of *Momordicacharantia*.

Keywords: Bioactive compounds; GC-MS; Nutritional attributes; Sewage sludge; Soil amendment.

Introduction

Momordicacharantia (with traditional names as Mitter melon,/bitter gourd), is a tropical and subtropical plant of the family cucurbitaceae. It is widely grown in India, South Asia, China, Africa and the Caribbean [1]. It is an annual climber. Bitter melon has been proved to contain several bioactive components with promising sugar regulatory effects and is being used in traditional medicines in Turkey [2]. The unripe fruits, seeds and aerial parts of bitter melon have been extensively used to treat diabetes [3]. It enhances the nutritional value of food as it contains several phenolic compounds and is used as a medicinal vegetable in many countries of the world [3]. In addition, this plant also used to banish intestinal gas, wound treatment, rheumatism and malaria. In Nigeria, Ghana and India the root of this plant is used as an abortifacient together with the fruit as well as an ingredient in aphrodisiac preparation [4]. Furthermore, fruits and leaves of the plant possess laxative, antibilious, emetic and stomachic effects [5]. Bioactive compounds such as anthocyanins, linoleic acid, rosmarinic acid, stigmasterol and ascorbic acid have been reported to effect living organisms positively by preventing them from free radical scavenging and oxidative stress [6].

Sewage sludge is a by-product of waste water treatment plants and is produced in large quantities worldwide [7]. Similar to other environmental issues, disposal of sewage sludge is a gigantic issue for the environmentalists across the globe [8]. Annual production of sewage sludge in various regions of the world has increased vigorously due to remarkable increase in population and, in turn increased urbanization and industrialization. In Europe, annual production of the sewage sludge has increased up to 8 million tonnes and in USA, it is reported to be 5.6 million tonnes. Same is the case for the other regions of the world. The cost to manage the sewage sludge is reported to be approximately 300$ per tonne. Several methods have been reported for its disposal. However, soil used for plant growth which is amended with sewage sludge has been reported as the safer and economical method of disposal [9]. Moreover, mixing of sewage sludge with soil significantly affect the concentration of bioactive components in the plant. It has been reported that total anthocyanins vary predominantly when grown in different soil systems. It can be deduced that the change in the soil quality as well as type may change the quantity of bioactive

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components in the growing plants [10]. Sewage sludge has been reported to contain many potential components such as high amount of nitrogen, phosphorus and organic matter. These constituents can enhance the fertility and quality of soil to achieve higher yields of the plants [11]. Hence, controlled mixing of sewage sludge may affect the nutritional potential of the plants and it may serve as a potent replacement of fertilizers used in agricultural practices [12].

The objective of present study is to optimize the soil with different ratios of sewage sludge in order to achieve promising nutritional and functional food attributes in *Momordicacharantia*. The instrument used for this purpose is GC-MS.

Experimental

Sample Collection

Samples of sewage sludge were obtained from the urban areas of district Sargodha, Pakistan and for this purpose; central drainage system of the city was selected. Central drainage system is a place where disposed waste material from all towns of the city is collected and waste water is being treated mechanically and physically. As a result of this treatment sewage sludge is produced. Sewage sludge was collected with the help of town municipality (TMA Sargodha) and transported to University College of Agriculture, Khushab road, University of Sargodha, Sargodha, Pakistan.

Preparation of Soil Amendments with Sewage Sludge

Experimental setup was designed using 18 earthen pots of same size (three replicates for each sewage sludge soil amendment, three for pure soil and three for pure sewage sludge). For this purpose, four various combinations of sewage sludge and soil (20% S.S, 40% S.S, 60% S.S, 80% S.S) were prepared along with pure soil and sewage sludge in separate containers (10 kg weight per pot) respectively, in the fields of University College of agriculture, Khushab Road, University of Sargodha, Sargodha. All soil amendments, pure soil and pure sewage sludge were taken in triplicates**.**

Extraction and pretreatment of fruit

The fruit grown on all plants in different sewage sludge soil amendments as well as in pure soil and pure sewage sludge was collected at the same time. Same weight of fruit from all pots was examined for this study. Three pieces of fruit from each pot were collected for triplicate analysis. All fruit was washed thoroughly and peeled off, mashed evenly by mechanical means in separate containers at room temperature and used for further analysis.

GC-MS analysis of Momordicacharantia (Fruit) extract

Analysis of *Momordicacharantia* (Fruit) samples was performed using GC-MS (QP2010 Shimadzu, Japan equipped with Chemstation GC-MS software). Helium (99.999%) was chosen as carrier gas. The flow rate of the carrier gas was maintained at 1.0 mL/min. All of the mass spectra were acquired in electron impact mode and ionization was performed at 70eV. To avoid solvent overloading, ionization was maintained off during first 3 minutes of each analysis. Injector temperature was 250ºC and oven temperature was 200ºC. Rate of heating was 10ºC rises per minute. Injection was performed in the split ratio of 200 and the volume of the sample was taken 10µL. All the samples were analyzed for 30 minutes through the column. The quadruple triple axis MS detector was set at 250°C. In almost all of the cases the retention time of all compounds was identical. All compounds were obtained from 19 to 25 minutes. NIST and Wiley database library installed in chemstation software of mass spectra were used for identification of bioactive compounds [13].

Results and Discussion

Growth of the plant and productivity of fruit

All plants grown were simultaneously monitored and highest growth rate was observed in case of plants growing in soil which was containing 60% sewage sludge. The fruit grown on plants being cultured in 60% sewage sludge soil amendment were slightly bigger and healthier compared to other soil amendments.

GC- MS Analysis of Momordicacharantia (Fruit) extract

Volatile components in the methanol extracts of the *Momordicacharantia* (Fruit) grown in pure soil and various sewage sludge amendments of soil were analyzed using GC-MS. Compound identification was performed by NIST (National Institute of Science & Technology) MS search 2.0 Library. Relative peak area calculations were used for the quantification of obtained compounds [14]. Selective bioactive components and relative peak areas of *Momordicacharantia* grown in soil with 20%, 40%, 60% and 80%amendment and pure sludge are shown in the Fig. 1 to 6 respectively. Fruit extracts of the plants obtained from six amendments showed variation in the quantity of the selective bioactive components. The compounds such as stigmasterol, oleic acid and 7, 11-hexadecadienal showed significant variation in the different amendments of soil and sewage sludge. Reviewing all the results it is obvious that the soil amendment with 60% sewage sludge showed the highest area under the curve for compounds under study. Stigmasterol, oleic Acid and 7,11-hexadecadienal are medicinally important compounds. For example, Stigmasterol is a steroid derivative and it is reported to have hypoglycemic properties along with cholesterol lowering ability. It also shows anticancer activities [15]. Oleic acid is a fatty acid and reported to reduce blood pressure, weight loss and prevents ulcerative colitis [16]. Similarly, 7,11-hexadecadienal is also an important secondary metabolite [17]. Marked variations in the concentration of these bioactives is associated with change in the characteristics of the soil. Reduction in concentration at higher ratios of sewage sludge such as 80% as well as in pure sewage sludge can be related to the presence of higher amounts of toxic heavy metals in the sewage sludge [18]. These heavy metals reported to reduce the growth of plants causing oxidative stress. Reactive oxygen species and free radicals generated due to these heavy metals affected the concentration of the secondary metabolites under study [19]. Therefore, high concentration of bioactive components was found in the soil amended with 60% sewage sludge. Thus, controlled and optimized amount of sewage sludge must be used to attain best outcome. Moreover, tolerance level of the plants relative to concentration of the sewage sludge varies from specie to specie [20]. Hence, optimization of sewage sludge mixing is required for any plant species to avoid drastic circumstances in terms of phytotoxicity and other ailments [21].

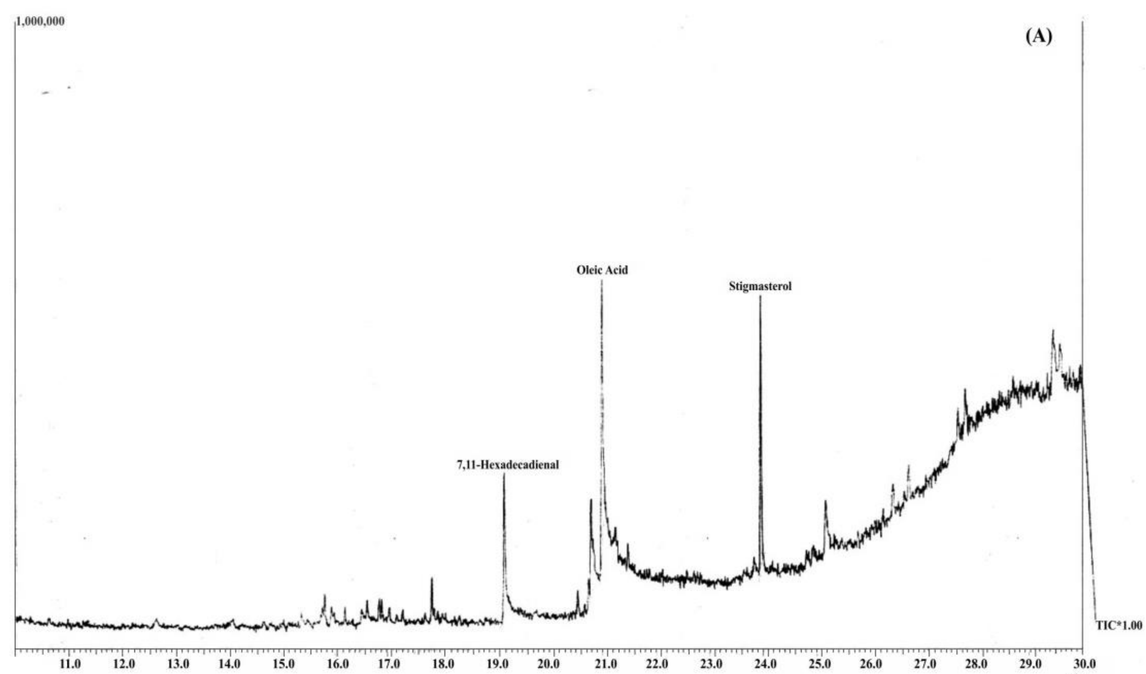

Fig. 1: GC-MS Analysis of *Momordicacharantia* L. (Fruit) Grown in Pure Soil (A).

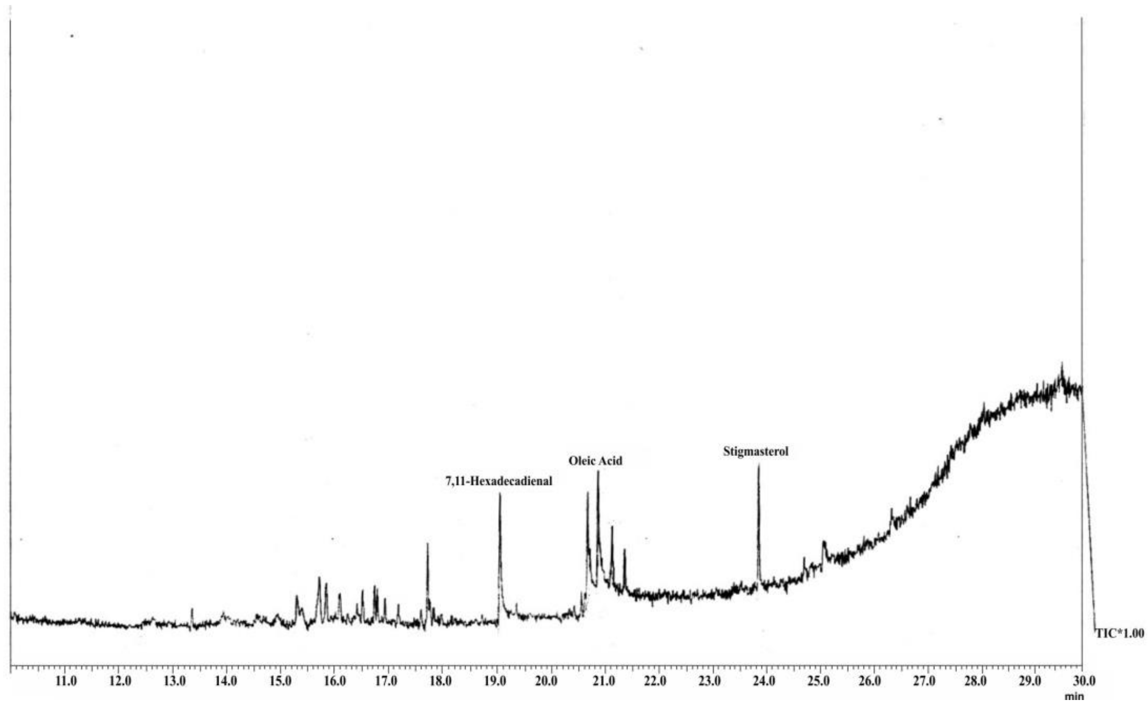

Fig. 2: GC-MS Analysis of *Momordicacharantia* L. (Fruit) Grown in Soil amended with 20% Sewage Sludge (B).

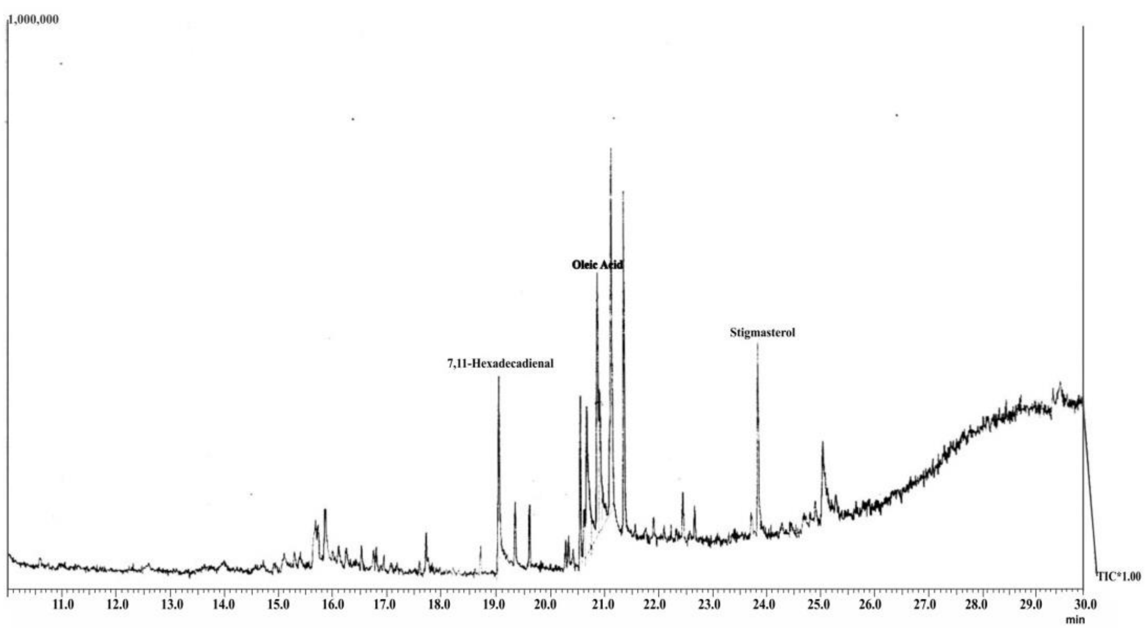

Fig. 3: GC-MS Analysis of *Momordicacharantia* L. (Fruit) Grown in Soil amended with 40% Sewage Sludge (C).

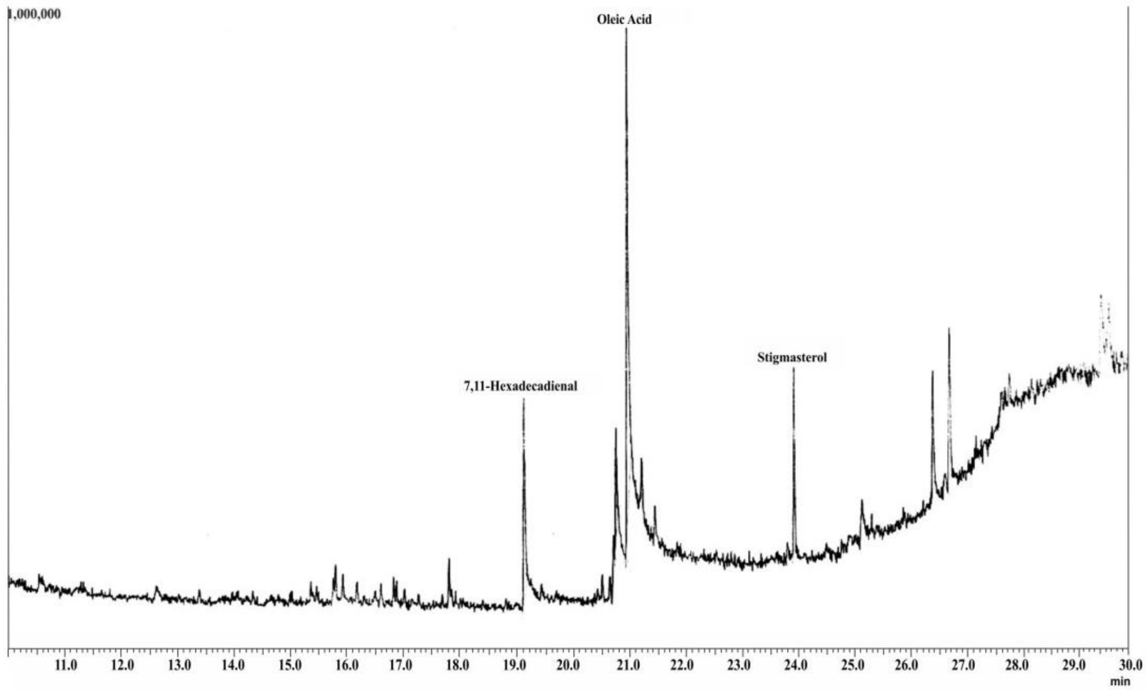

Fig. 4: GC-MS Analysis of *Momordicacharantia* L. (Fruit) Grown in Soil amended with 60% Sewage Sludge (D).

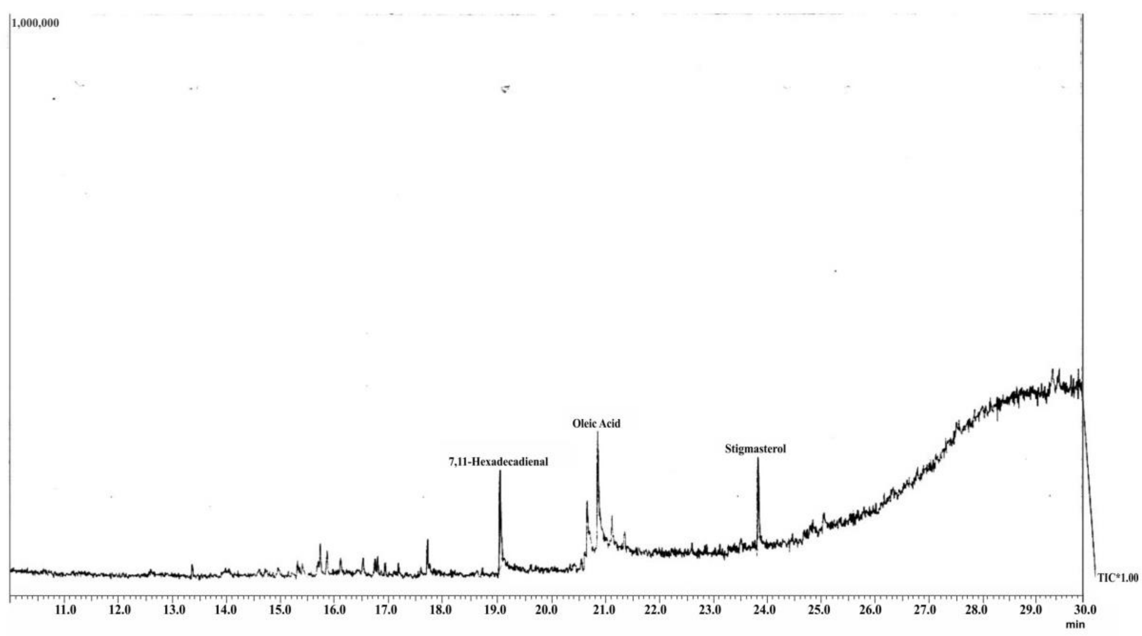

Fig. 5: GC-MS Analysis of *Momordicacharantia* L. (Fruit) Grown in Soil amended with 80% Sewage Sludge (E).

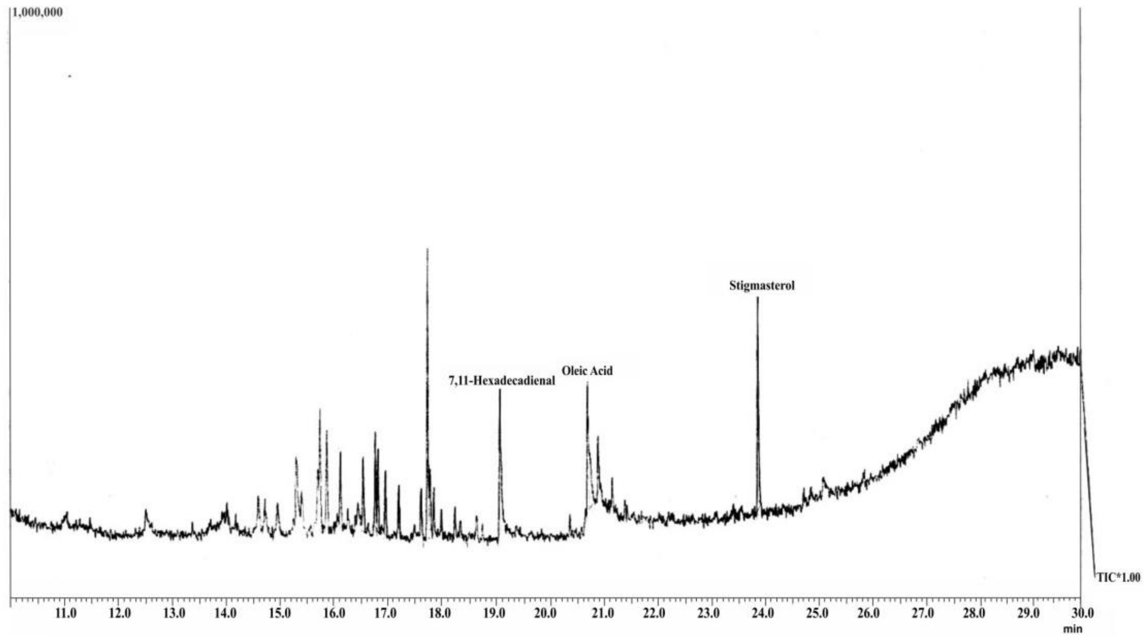

Fig. 6: GC-MS Analysis of *Momordicacharantia* L. (Fruit) Grown in Pure Sewage Sludge (F).

Table-1: GC-MS analysis of Momordicacharantia (fruit) grown in pure and amended soil.

Soil and sewage sludge amendments	Peak Area			
	Stigmasterol	Oleic Acid	7,11-Hexadecadienal	Dried food sample
Pure soil	272095	323573	361742	mg/g
Soil amended with 20% Sewage Sludge	342145	372575	432441	Sigmasterol= 24 Oleic acid=44 7,11-hexadecadienal=91
Soil amended with 40% Sewage Sludge	431164	414234	428432	Sigmasterol= 48 Oleic acid=80 7,11-hexadecadienal=106
Soil amended with 60% Sewage Sludge	485173	455167	484673	Sigmasterol= 140 Oleic acid=105 7,11-hexadecadienal=189
Soil amended with 80% Sewage Sludge	424166	450071	442564	Sigmasterol= 180 Oleic acid=76 7,11-hexadecadienal=112
Pure Sewage Sludge	372621	370354	411473	Sigmasterol= 70 Oleic acid=39 7,11-hexadecadienal=88

Conclusion

Management of sewage sludge is a worldwide concern as it is being produced in immense amount due to the increase in population, urbanization and rapid industrialization. Hence, present study provides a solution for the safer and economical disposal of the sewage sludge. Moreover, sewage sludge amendment resulted in enhancement of the potential bioactive components like 7,11-Hexadecadienal, Oleic Acid and Stigmasterol in *Momordicacharantia* L. Presence of toxic metals and polycyclic aromatic hydrocarbons in the sewage sludge limits its utility for the cultivation purposes. Therefore, controlled amount of sewage sludge was used to get maximum benefits from the potential of sewage sludge rather its toxic ailments. Significant variations of bioactive components were found in all the plants and soil amended with 60% sewage sludge provide maximum amount of the components under study. Hence, it was found that mixing of sewage sludge is beneficial to attain the promising nutritional attributes in *Momordicacharantia* L. plant.

References

1. M. Gupta, S. Sharma, A. K. Gautam and R. Bhadauria, *Momordica charantia* linn.(Karela): Nature’s Silent Healer, *Int. J. Pharm. Sci. Rev. Res.*,**11**, 32 (2011).
2. S.N. Jana, P.M. Mazumder and G. Nagaraju, *Momordica Charantia* linn As A Medicinal Plant, (2017).

3. R. Shukia, S. Sharma, D. Puri, K. Prabhu and P. Murthy, Medicinal Plants for Treatment of Diabetes Mellitus, *Ind. J. Clin. Biochem.,* **15**, 169 (2000).
4. R. Bakare, O. Magbagbeola and O. Okunowo, Nutritional and Chemical Evaluation of *Momordica Charantia*, *J. Med. Plant. Res*., **4**, 2189 (2010).
5. J. Kubola and S. Siriamornpun, Phenolic Contents and Antioxidant Activities of Bitter Gourd (*Momordica charantia* L.) Leaf, Stem And Fruit Fraction Extracts In Vitro, *Food Chem.*,**110**, 881 (2008).
6. J. Banerjee, R. Singh, R. Vijayaraghavan, D. MacFarlane, A.F. Patti and A. Arora, Bioactives from Fruit Processing Wastes: Green Approaches to Valuable Chemicals, *Food Chem.*,**225**, 10 (2017).
7. C. Baronti, R. Curini, G. D'Ascenzo, A. Di Corcia, A. Gentili and R. Samperi, Monitoring Natural and Synthetic Estrogens at Activated Sludge Sewage Treatment Plants and in a Receiving River Water, *Environ. Sci. Technol*., **34**, 5059 (24) (2000).
8. M. Khwairakpam and R. Bhargava, Vermitechnology for Sewage Sludge Recycling, *J. Hazard. Mat*.,**161**, 948 (2009).
9. K. Raina, D. Kumar and R. Agarwal, Promise of Bitter Melon (*Momordica charantia*) Bioactives in Cancer Prevention and Therapy, *Seminars in Cancer Biology*.,**40**,116, (2016).
10. S.M. Paunović, P. Mašković, M. Nikolić and R. Miletić, Bioactive Compounds and Antimicrobial Activity of Black Currant (*Ribes nigrum* L.) Berries and Leaves Extract Obtained by Different Soil Management System, *Sci. Horti*.,**222**, 69 (2017).
11. R. Singh and M. Agrawal, Potential Benefits and Risks of Land Application of Sewage Sludge, *Waste Manage*.,**28**, 347(2008).
12. V. Arthurson, Closing the Global Energy and Nutrient Cycles Through Application of Biogas Residue to Agricultural Land–Potential Benefits And Drawback, *Energies*.,**2**, 226 (2009).
13. J. Quintana, I. Martí and F. Ventura, Monitoring of Pesticides in Drinking and Related Waters in NE Spain With a Multiresidue SPE-GC–MS Method Including an Estimation of the Uncertainty of the Analytical Results, *J. Chromato. A*.,**938**, 3 (2001).
14. Sharma, P. K. Rai and S. Prasad, GC–MS Detection and Determination of Major Volatile Compounds in *Brassica Juncea*L. Leaves and Seeds, *Microchem. J.*, 138 (2018) 488-493.
15. D. Rathee, S. Rathee, P. Rathee, A. Deep, S. Anandjiwala and D. Rathee, HPTLC Densitometric Quantification of Stigmasterol and Lupeol from *Ficus Religiosa*, *Arabian J. Chem*. **8**, 366 (2015).
16. J.W. Alexander, Immunonutrition: the Role of ω-3 Fatty Acids, *Nutrition*.,**14**, 627 (1998).
17. M.R. Tabilio, D. Fiorini, E. Marcantoni, S. Materazzi, M. Delfini, F. R. De Salvador and S. Musmeci, Impact of the Mediterranean Fruit Fly (Medfly) *Ceratitis Capitata* on Different Peach Cultivars: the Possible Role of Peach Volatile Compounds, *Food Chem*., **140**, 375 (2013).
18. K. E. Giller, E. Witter and S.P. Mcgrath, Toxicity of Heavy Metals to Microorganisms and Microbial Processes in Agricultural Soils: A Review, *Soil Biol. Biochem*., **30**, 1389 (1998).
19. S.M. Gallego, M. P. Benavides and M.L. Tomaro, Effect of Heavy Metal Ion Excess on Sunflower Leaves: Evidence for Involvement of Oxidative Stress, *Plant Sci*., **121**, 151 (1996).
20. G. Zhang, M. Fukami and H. Sekimoto, Influence of Cadmium on Mineral Concentrations and Yield Components in Wheat Genotypes Differing in Cd Tolerance at Seedling Stage, *Field Crop. Res*., **77**, 93 (2002).
21. P. Miralles, T. L. Church and A.T. Harris, Toxicity, Uptake, and Translocation of Engineered Nanomaterials in Vascular Plants, *Environ. Sci. Technol*., **46**, 9224 (2012).