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Influence of municipal waste treated sludge on the quality attributes of African marigold (*Tagetes erecta*)

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Abstract

Using treated sludge to amend soil in agriculture presents opportunities and challenges, particularly concerning the augmentation of heavy metals in cultivated plants. This research looks into how applying treated sludge affects the build up of heavy metals in African marigold (*Tagetes erecta*), a popular ornamental plant that could potentially help with phytoremediation. Several treatment combinations were imposed, out of which treated sludge at 2.5 t/ha showed promising results. The effect of incorporating treated sludge was found to be better in terms of using conventional manures like Farm Yard Manure and Vermicompost in quantitative terms. The sludge processed at the treatment plant underwent strict treatment procedures, resulting in odourless material that can be used as an organic amendment with nutrients. Heavy metal concentrations in plant tissues were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICPMS) to assess the uptake and accumulation of heavy metals by African marigolds. Results indicated that treated sludge application did not significantly influence the accumulation and bioavailability of heavy metals in African marigolds. The treated sludge helped plants grow better and absorb nutrients; it did not cause higher levels of harmful heavy metals like cadmium and lead in the plants. The levels of heavy metals found were lower than what is considered unsafe for plants. In addition, treated sludge can serve as a soil conditioner to enhance the physicochemical properties of the soil.

Keywords

Treated sludge; African marigold; heavy metal; soil conditioner

Introduction

Treated sludge, a by product of waste treatment plants, has garnered significant attention as a potential soil enhancer and organic fertilizer owing to its elevated nutrient substance and organic matter composition. Applying municipal waste-treated sludge as a soil conditioner could influence crops' growth, yield and quality attributes in numerous ways. Firstly, the nutrient composition of sludge, particularly its high nitrogen, Phosphorus and potassium content, could enhance plant height and biomass accumulation, ultimately impacting flower yield. Secondly, the organic matter in sludge may improve soil structure, water-holding capacity, and aeration, creating a favourable environment for root development and nutrient uptake. However, the prevalence of heavy metals, pathogens and other contaminants in sludge necessitates careful evaluation of its effects on various aspects of plant growth and quality.

African marigold (*Tagetes erecta*), a popular ornamental plant, is widely cultivated for its vibrant flowers. It is rich in carotenoids, flavonoids and essential oils, contributing to their aesthetic appeal and medicinal properties. The heavy metals present in sludge, such as cadmium, lead and chromium, could accumulate in the plant tissues, propounding perils to human and environmental well-being and potentially altering the biosynthesis and accumulation of secondary metabolites responsible for the quality attributes of African marigold flowers. Furthermore, the potential presence of organic contaminants like pharmaceutical and nutraceutical compounds in sludge may have unintended consequences on plant physiology and biochemistry. An investigation of the influence of treated sludge on the growth, yield and quality attributes of African marigolds was carried out to assess the suitability of treated sludge as a soil amendment and to ensure the safety and quality of the flower produced. This study aimed to provide comprehensive insights into the effect of sludge application in African marigolds, ultimately contributing to the sustainable and responsible utilization of this valuable resource in horticultural practices and as a potential nutrient supplier and soil amendment for marigold cultivation.

Numerous studies have evaluated the effects of sewage sludge incorporation on soil properties and crop productivity. A review highlighted the beneficial impacts of sewage sludge on soil fertility, water-holding capacity and microbial activity. They reported increased yields of cereals, vegetables and forage crops when grown in sludge -amended soils compared to inorganic fertilizers or unamended control soils (1). The long-term effects of anaerobically digested sewage sludge on the properties of soil and *Triticum aestivum* L. yield over ten years were analyzed. It was found that sludge application significantly improved the organic matter content of the soil, cation exchange capacity and availability of nutrients, leading to higher wheat grain yield than mineral fertilization. However, the accumulation of heavy metals, particularly cadmium, was also observed in the soil and wheat grains, necessitating careful monitoring and management (2).

A field study observed that applying composted sewage sludge to degraded Mediterranean soil enhanced soil organic carbon, nitrogen and phosphorus levels, microbial biomass, and activity. The sludge compost also improved the growth and yield of ryegrass (*Lolium multiflorum* Lam.) and oat (*Avena sativa* L.) grown on amended soil (3). The potential risks associated with heavy metals, persistent organic pollutants (POPs) and pathogens in sewage sludge were reviewed. Appropriate sludge treatment processes such as anaerobic digestion, composting and thermal drying were emphasized to reduce contaminant levels and meet regulatory standards for land application (4).

A study investigated the effects of municipal sewage sludge on the quantitative characteristics and quality of African marigolds. Low doses of sludge (10-20%) enhanced plant growth parameters like plant height, Number of branches, flowers per plant and flower diameter compared to control. However, higher sludge doses (>30%) had inhibitory effects. The increased growth at low sludge levels was attributed to the supply of plant nutrients, while inhibition at high levels may have been caused by heavy metal phytotoxicity (5). On the other hand, some studies have reported the harmful influence of sewage sludge application on African marigold quality. The impact of fertilizing sewage sludge from different wastewater treatment plants on marigolds grown in a greenhouse was also studied. It was observed that sludge application resulted in reduced germination, seedling growth and flowering compared to the control treatment with inorganic fertilizers. The adverse effects were more pronounced with increasing sludge doses and were partly attributed to plant stress from elevated heavy metal concentrations, particularly chromium and nickel (6). The impacts of composted municipal solid waste on growth, flowering and accumulation of heavy metals and persistent organic pollutants in African marigolds were investigated in a study. The compost enhanced growth parameters like plant height and Number of flowers at low doses (10-ton ha^{-1}). The higher dose of >20ton ha^{-1} inhibited growth and flowering. The plants also accumulated significant heavy metals like cadmium, chromium, copper, nickel, lead, zinc and polycyclic aromatic hydrocarbons in their shoots, posing potential health risks (7).

The accumulation of heavy metals *viz*., Cadmium, Chromium, Copper, Nickel, Lead and Zinc) in different plant parts of radish (*Raphanus sativus* L.) grown in soil amended with increasing doses of sewage sludge were evaluated. It was found that heavy metal concentrations in the edible roots increased significantly with higher sludge application rates exceeding permissible limits for Cadmium, Nickel and Lead at the highest dose. However, the origins accumulated lower metal levels than the shoots and leaves. Similar trends were observed for lettuce (*Lactuca sativa* L.) cultivated in sewage sludge-amended soil, with metal accumulation following the order: roots < stems < older leaves < younger leaves (8).

The range of heavy metal absorption in plants depends on soil properties, pre-treatment methods of sludge and plant species/cultivars. For instance, in a field study, it was observed that the accumulation of Cadmium, Copper, Nickel, Lead and Zinc in rapeseed - *Brassica napus* L. was significantly lower when grown in soils amended with thermally dried sewage sludge compared to anaerobically digested sludge. This was attributed to the higher metal availability in the latter treatment (9).

Using biochar as a soil amendment to immobilize heavy metals and reduce their bioavailability to plants grown in sludge-amended soils was investigated. It was found that biochar effectively reduced the uptake of cadmium and lead by radish (*Raphanus sativus* L.) cultivated in sewage sludge-treated soil (10).

With this background, this research was carried out to evaluate the impact of treated sludge as an amendment for growth in African marigolds and its potential ways of accumulating macro and microelements and heavy metals. The application of soil amendments like biochar, zeolites, or organic matter to immobilize metals, selection of low-accumulating cultivars and phytoremediation using metal-accumulating plant species are effectively followed now (11). Additionally, proper sludge treatment and quality control measures are essential to reduce metal loading in sludge before land application.

Judicious application of municipal sewage sludge at low doses benefits growth and flowering attributes by supplying essential plant nutrients. However, excessive sludge doses or sludge contaminated with high levels of heavy metals and organic pollutants may adversely impact quality due to phytotoxicity and accumulation of toxic substances in plant tissues. Thus, the careful monitoring of sludge quality and controlled application rates are necessary to derive the benefits of sludge as a soil amendment while avoiding potential risks to plant growth and quality.

Materials and Methods

Experimental site and geography

This study was conducted at Floriculture Research Station, Tamil Nadu Agricultural University, Thovalai, Kanyakumari Dt., Tamil Nadu, India, at a longitude of 8.2312° N and latitude of 77.5060° E and an elevation of 81 meters above mean sea level.

Treatment with treated sludge

The current study was conducted for two cropping seasons from March 2023 - June 2023 and July 2023 - October 2023. African marigold seeds were sown in protrays for germination and transplanted in the field 25 days after sowing at a 45 × 35 cm spacing. The field for planting was thoroughly ploughed to a fine tilth. The nutrient availability in the soil was analyzed before planting (Fig 1a. 1b. 1c). The treated sludge was also analyzed for nutrient availability and the presence of heavy metals (Fig 1d, 1e). During the last ploughing, 9 treatments were imposed with different combinations. This experiment was laid out in a Randomised Block Design with nine treatments. T1 - Absolute control (FYM 25t/ha), T2 - FYM 25t/ha + Recommended Dose of Fertilizer (RDF) 90:90:75 kg NPK/ha, T3 - Treated sludge -2.5 t/ha, T4 - Treated sludge - 5.0 t/ha, T5 - RDF (90:90:75 kg NPK/ha) + Treated sludge 2.5t/ha, T6 - RDF (90:90:75 kg NPK/ha) +Treated sludge 5 t/ha, T7 - RDF (90:90:75 kg NPK/ha) + Treated sludge 2.5t/ha + Micronutrients ($FeSO_4$ 0.5% + $ZnSO_4$ 0.5%), T8 - RDF (90:90:75 kg NPK/ha) + Treated sludge 5.0t/ha + Micronutrients ($FeSO_4$ 0.5% + $ZnSO_4$ 0.5%) and T9 - RDF (90:90:75 kg NPK/ha) + Micronutrients ($FeSO_4$ 0.5% + $ZnSO_4$ 0.5%). The treated sludge was incorporated into the soil per the treatment specifications to evaluate its performance in enhancing quality attributes in African marigolds (*Tagetes erecta*).

Sample details:

Out of the treatments mentioned above, two samples were selected for analysis. S1 (Control) - Recommended dose of fertiliser (90:90:75 kg NPK/ha) + micronutrients ($FeSO_4$ 0.5% + $ZnSO_4$ 0.5%) and S2 - Recommended dose of fertiliser (90:90:75 kg NPK/ha) + Treated sludge 2.5t/ha + micronutrients ($FeSO_4$ 0.5% + $ZnSO_4$ 0.5%)

The treatment S2 - Recommended dose of fertilizer (90:90:75 kg NPK/ha) + Treated sludge 2.5t/ha + micronutrients ($FeSO_4$ 0.5% + $ZnSO_4$ 0.5%) were selected based on its best performance in terms of morphological growth amongst the other treatments. This was compared with control - S1, which recommended a dose of fertilizer (90:90:75 kg NPK/ha) + micronutrients ($FeSO_4$ 0.5% + $ZnSO_4$ 0.5%). The plants grown under control treatment were not imposed with treated sludge. Hence, the control was selected to isolate the addition of treated sludge in S2, serving as a baseline to represent the standard condition of the experiment. The leaf samples were collected during the active growth stage of the plant, 60 days after transplanting. The fresh leaf sample from the third growing tip was collected, sun-dried and powdered to assess heavy metal accumulation.

Sample Preparation for Chemical Analysis

The multi-element Aqueous Certified Reference Material (CRM) is a mixture of 30 elements, Silver, Aluminium, Arsenic, Boron, Barium, Beryllium, Calcium, Cadmium, Cobalt, Chromium, Cesium, Copper, Iron, Mercury, Potassium, Lithium, Magnesium, Manganese, Molybdenum, Sodium, Nickel, Phosphorus, Lead, Antimony, Silicon, Tin, Titanium, Thallium, Vanadium, Zinc 100 µg/ml in 5 % HNO_3 was procured from VHG Labs, Manchester, NH, USA (12). Nitric acid (HNO_3) was procured from Fisher Chemicals, India, at 67 -70 % concentration for trace metal analysis. High-performance liquid chromatography (HPLC) grade water is used to Prepare all samples and standards (13). All lab accessories used were of "A" grade quality and duly calibrated. The calibrated micropipettes were used with the range of two µL to 1000 µL (14).

Inductively Coupled Plasma-Mass Spectrometry

Inductively Coupled Plasma-Mass Spectrometry [ICPMS] was used to quantify the heavy metal accumulation in the African marigold leaf samples. ICPMS - Thermo Scientific™ iCAP™ RQ was equipped with micro mist borosilicate glass nebulizer, quartz cyclonic spray chamber, ICP torch, nickel sampler cone and skimmer cone, Quadrapole mass analyzer and mass spectrometry detector (15). The two leaf samples were analyzed in Kinetic Energy Discrimination (He KED) mode using pure Helium as the collision gas in the collision/reaction cell (CRC) under optimized auto-tune conditions of the equipment directly from Quality control with Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software (16). The sampling process was automated using ASX 560Auto sampler from Omaha,

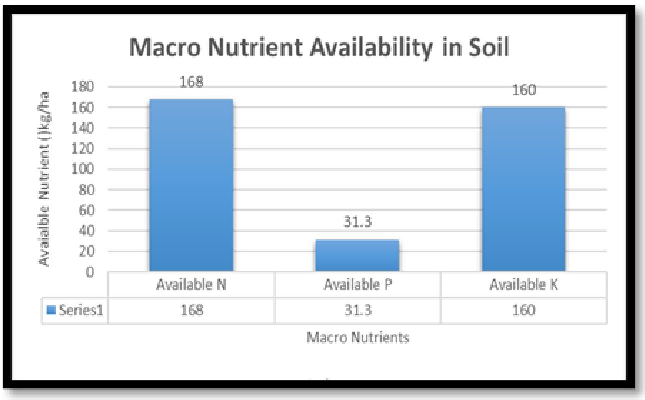

Fig. 1a. Available Macro Nutrients in Soil

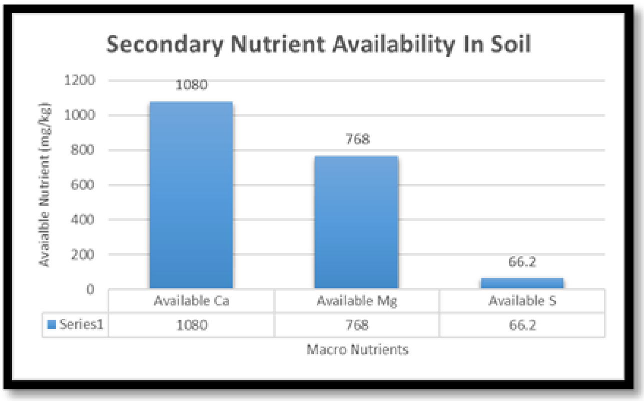

Fig. 1b. Available Secondary Nutrients in Soil

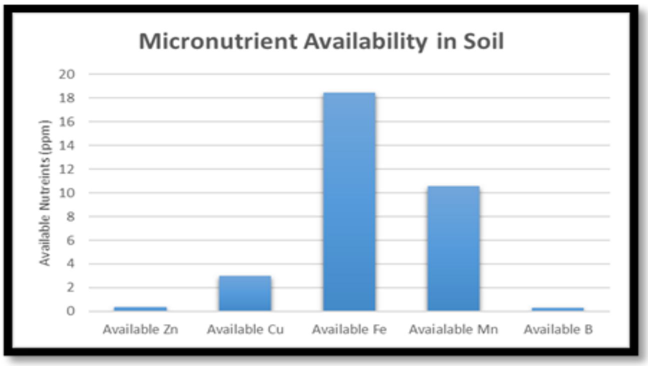

Fig. 1c. Available Micro Nutrients in Soil

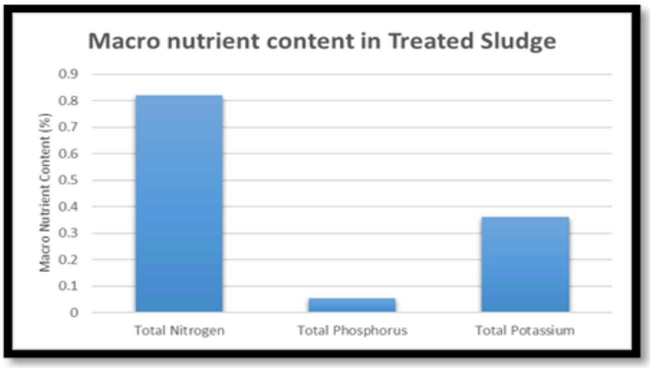

Fig. 1d. Available Macronutrients in Treated Sludge

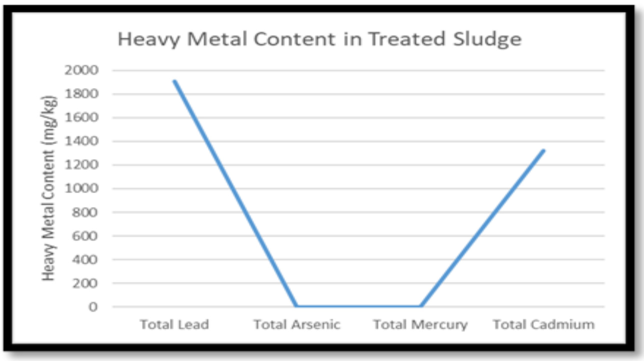

Fig. 1e. Presence of Heavy Metals in Treated Sludge

NE, USA. A closed-vessel microwave digestion system (Multi-wave GO Anton Paar) with a multi-wave pro rotor with temperature and pressure sensor and provided with an auto pressure vent Poly Tetra Fluro Ethylene PTFE vessel was utilized for digestion of the sample (17).

The standard calibration solutions were prepared by accurately measuring one ml of mixed standard reference solution and pipetting it into a 100 mL volumetric flask. Then, the solution was diluted to 100 mL using HPLC-grade water. This solution was taken as the stock used to prepare standards for calibrations and storage. Then appropriate aliquots were taken and further diluted with 5% nitric acid in HPLC grade water to give a series of calibration standard solutions with the concentration range of 1.0, 20.0, 50.0 and 100.0 µg/L (18).

The sample solutions were prepared from accurately weighed, dried homogenized powder samples already milled with Teflon mortar to avoid metal contamination and of weight 0.20 ± 0.01 g (19). Dried leaf samples were taken in 6.0 mL of concentrated ultra-pure Nitric acid in a tightly closed Poly Tetra Fluro Ethylene PTFE vessel and digested in a microwave digester. The digestion was carried out in three steps with a constant microwave power. The program was set to increase temperature to 160°C in 10 minutes and held at that for five minutes. Then, the digester was cooled down at room temperature. The digested samples were diluted with HPLC-grade water up to 50 ml (20). Three replicate samples were prepared in each sample for analysis.

The Operating conditions for Inductively Coupled Plasma Mass Spectrometry (ICPMS) are listed below. Radio Frequency (RF) power (W) - 1500 (2), Nebuliser pump (rpm) - 40, Plasma gas flow rate (L min^{-1}) - 14, Extract lens (V) - 1.5, Auxiliary gas flow rate (L min^{-1}) - 0.8, Pirani pressure (mbar) - 1.81, Carrier gas flow rate (L min^{-1}) - 1, Penning pressure (mbar) - 3.05x10-7, Spray chamber *T* (°C) - 2, Plasma exhaust (mbar) - 0.45-0.56, Sample depth (mm) - 8.6, Number of replicates - 3, RF power (W) - 1500 (15,17,21,22,23).

Results and Discussion

The outcomes achieved using the Helium collision technique in Inductively Coupled Plasma Mass Spectrometry - ICPMS are furnished in Fig. 2a, Fig. 2b, Fig. 2c and Fig. 2d. The accurate amount of micro and macronutrient uptake by plants are shown in parts per million. The data provided represent the elemental composition of the African marigold leaf samples collected 60 days after transplanting and grown in soil amended with treated sludge. The concentrations of various elements are given in parts per million (ppm). To provide a comprehensive summary and analysis, it is essential to understand the optimal levels of these elements and heavy metals for plant growth and potential environmental implications.

Nutrient status of the experimental soil

The nutrient availability in the soil collected from the experimental field is furnished in Fig. 1a, Fig. 1b, Fig. 1c and Fig. 1d. The initial Nitrogen content in the soil was 168 kg/ha, while the available Phosphorus content in the soil was 31.3 kg/ha. The available Potassium content in the soil was 160 kg/ha.

Impact of treated sludge on leaf macronutrients

The elemental availability in the African marigold leaves is presented in Fig. 2a, Fig. 2b, Fig. 2c and Fig. 2d. In the present study, the concentration of Calcium in S1 Control, without any sludge application was 4148.41 ppm, whereas in S2 was 7287.466 ppm. Moreover, this concentration level falls in the optimal category with a typical range from 5,000 to 20,000 ppm in plant leaves (24). Adding sludge has enhanced adequate calcium levels necessary for proper plant growth and development. Calcium plays crucial roles in cell wall structure, membrane permeability and enzyme activation (24). It is also involved in signalling pathways and stress responses. The Potassium concentration in the leaves of untreated samples was 11345.3 ppm, while in the leaves applied with treated sludge at 2.5t/ha, it was 17,595.871 ppm. It falls under the optimal level, which varies between 10,000 to 30,000 ppm for most plant leaves (25,26). Potassium plays vital roles in enzymatic activation, protein synthesis, photosynthesis and osmoregulation. It is also involved in stomatal regulation, transport of nutrients and stress tolerance (26). It was highly evident that the sludge application was reflected in increased Potassium content. The magnesium content of leaves in control was 5142.41 ppm, while that of leaves treated with sludge was 9,328.822 ppm. This is significantly higher than the leaves' optimal range of 2,000 to 6,000 ppm. Magnesium is a critical component of the chlorophyll molecule and is essential for photosynthesis, enzyme activation, carbohydrate metabolism and the stabilization of ribosomes and nucleic acids. However, excess Magnesium interferes with the uptake of calcium and potassium. Since the Calcium and Potassium content was high in the current study, it can be inferred that the excess Magnesium did not affect the elemental prevalence and availability of the two elements. The concentration of Phosphorus in the control leaf sample was 1891.07 ppm, while in the leaf sample collected from the plant treated with treated sludge at 2.5 t/ha was 3,333.366 ppm, which falls under the optimal range of 1,000 to 4,000 ppm for plant leaves (27). So, it was evident that the application of treated sludge at 2.5t/ha is beneficial and has increased the Phosphorus content of the leaves. Phosphorus is an essential macronutrient that plays crucial roles in energy transfer, nucleic acid synthesis, membrane structure and enzyme regulation (27). Excessive Phosphorus can lead to nutrient imbalances and potentially contribute to environmental issues like the eutrophication of water bodies. However, in this experiment, the nutrient imbalances due to excess Phosphorus did not arise as the phosphorus content was within the optimal range.

Impact of treated sludge on leaf micronutrients

The boron level in the leaves at 60 days after transplanting in the control leaf sample was 32.32 ppm. The leaves of

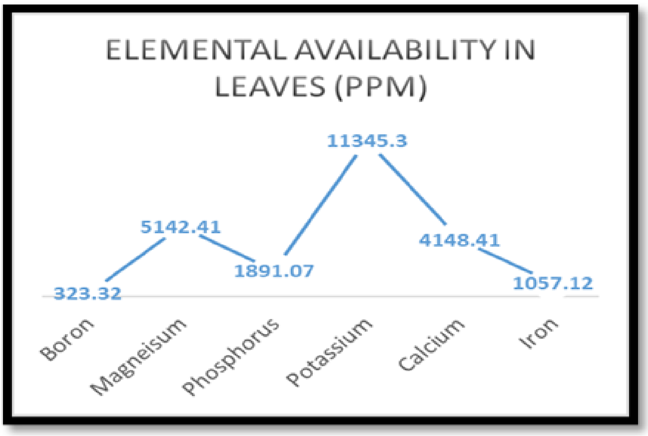

Fig. 2a. Elemental Availability in African marigold Leaves – S1 (ppm)

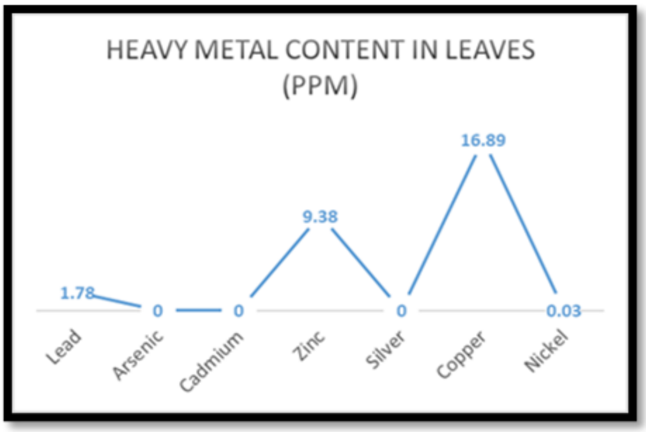

Fig. 2b. Heavy Metal Content in African Marigold Leaves – S1 (ppm)

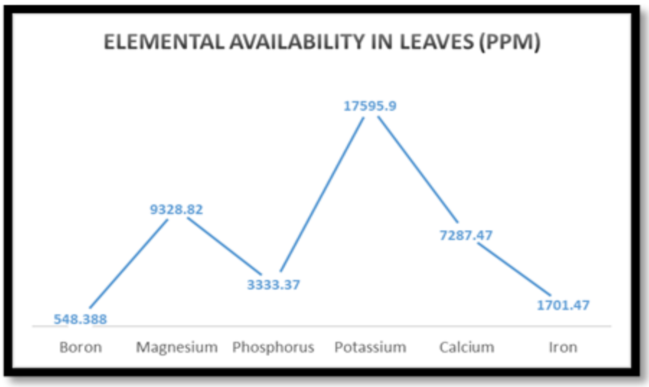

Fig. 2c. Elemental Availability in African marigold – S2 Leaves (ppm)

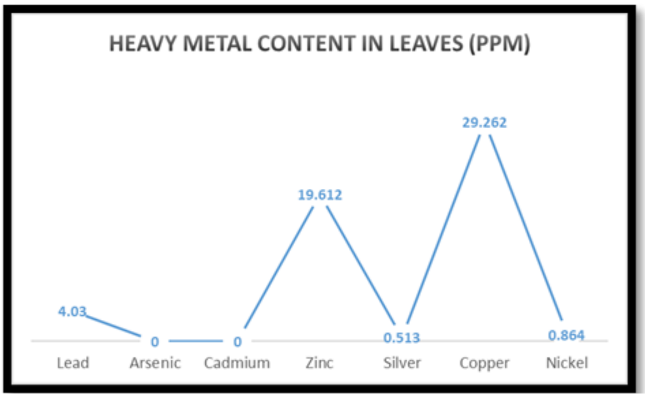

Fig. 2d. Heavy Metal Content in African Marigold Leaves – S2 (ppm)

plants treated at 2.5t/ ha of treated sludge were 54.8388 ppm, which is well within the optimal range of 20 to 100 ppm found in plant leaves (26). The iron concentration in control was also within the acceptable range in control at 1057.12 ppm and in the treated sample was 1,701.474 ppm. The optimal level of iron content in plant leaves is 50 to 5,000 ppm. In the present study, the treated sludge increased the iron content. The zinc concentration in the control group was 9.38 ppm, while in the treated leaf sample, it was 19.612 ppm and it was within the typical range of 15 to 100 ppm found in plant leaves (26).

Impact of treated sludge on accumulation of heavy metals on leaves

The concentration of heavy metals, *viz.,* Lead, Arsenic and Cadmium, was analyzed in the marigold leaves 60 days after transplanting in the plants, and 2.5 t/ha of treated sludge was applied. The results were compared with those grown in control plots without sludge application. The lead concentration of the leaf sample in the control was 1.78 ppm, and in leaves from plants incorporated with treated sludge, it was 4.030 ppm. This is considered low for plant leaves; the typical range is below ten ppm (24). Lead is a non-essential heavy metal regarded as toxic to plants as plants' tolerance level of lead is only 10 ppm. Even at a minimal higher range than the optimal level, lead can interfere with various physiological processes in plants, such as photosynthesis, respiration and enzyme activities (28). It can also disrupt cell membrane integrity and cause oxidative stress. Lead accumulation in edible plant parts can pose health risks to humans and animals through food (24).

The data on arsenic content of leaves indicates that arsenic was not present in both treatments, which is a positive finding as arsenic is a carcinogenic heavy metal. The typical range for arsenic in plant leaves is below 1 ppm. Arsenic is another non-essential and highly toxic heavy metal for plants. It is reported that arsenic can inhibit plant growth, disrupt nutrient uptake metabolism and induce oxidative stress (29). Arsenic can accumulate in plant tissues, particularly in the roots, and its presence in edible parts raises food safety concerns (24). Like arsenic, cadmium was also reported as "Nil," as its concentration is below the detection limit. Cadmium is a highly toxic heavy metal, and its absence is highly desirable. The typical range for cadmium in plant leaves is below 0.5 ppm. Cadmium is a non-essential and highly toxic heavy metal for plants. Cadmium can interfere with various physiological processes, including photosynthesis, respiration, and enzyme activities (29). Cadmium can disrupt water and nutrient uptake, leading to stunted growth and leaf chlorosis (30). Its accumulation in edible parts of plants poses significant health risks to humans and animals (24). So, as the Cadmium content in the leaf sample of the treatment-imposed field is reported as Nil, it is inferred that the treated sludge application is safer for marigolds regarding plant health and the environment.

Influence of treated sludge on the presence of other elements in leaves

Lab analysis estimated the elemental content of the marigold leaves imposed with treated sludge (S2) 60 days after planting. The results revealed that the sodium concentration of the leaves was 236.978 ppm. This is within the typical range of 100 to 1,000 ppm in plant leaves (26). The copper concentration of 29.262 ppm was within the optimal range of 5 to 30 ppm for plant leaves (24). The nickel level of the leaf sample at 0.864 ppm from the plant imposed with treated sludge was within the typical range of 0.1 to 5 ppm found in plant leaves (31). As reported in some studies, the silver concentration of 0.512 ppm is within the typical range of 0.1 to 1 ppm found in plant leaves. However, silver is not an essential nutrient for plants (32). According to some studies, the titanium level was relatively high at 145.694 ppm compared to the typical range of 10 to 50 ppm found in plant leaves. However, titanium is generally considered non-toxic to plants at low levels (33). The vanadium concentration was 2.697 ppm and is within the typical range of 0.5 to 5 ppm found in plant leaves. As reported in various studies, the chromium level of 4.263 ppm was slightly higher than the typical range of 0.5 to 3 ppm found in plant leaves. However, the effect of toxicity is negligible (34). In the present study, the treated sludge application at 2.5t/ha does not highly influence the prevalence of other elements in the leaves.

Conclusion

The present study concluded that treated sludge at 2.5t/ha can be safely and effectively used for marigold. It enhanced the nutrient availability in the soil, leading to improved growth, flowering and biomass accumulation in African marigold plants. The treated sludge supplied essential macronutrients, such as Phosphorus and potassium, and micronutrients, such as iron, manganese, and zinc. Overall, it can be concluded that the African marigold grown in soil amended with treated sludge has elevated levels of macronutrients, including Magnesium and potassium. The uptake of these nutrients by leaves is evident, and the elevated concentrations benefitted marigold growth. While sewage sludge can provide valuable nutrients for plant growth, its application to agricultural soils may lead to the accumulation of heavy metals in various plant parts at excessive sludge doses. Hence, it is necessary to optimize crop-specific dosages of treated sludge for safe utilization to ensure food safety and prevent environmental contamination when using it as a soil amendment.

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Authors' contributions

KPP carried out the experiment, took observations and analyzed the data. **SR** guided the research by formulating the concept, helped secure funds, and approved the final manuscript. **KLD** contributed by developing the ideas, reviewing the manuscript, and helping procure research grants. **SA** contributed by imposing the experiment and helped edit, summarise and revise the manuscript. **MS** helped summarize and revise the manuscript. **MP** helped edit, outline, and modify the manuscript**.**

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

Declaration of generative AI and AI-assisted technologies in the writing process

During the Preparation of this work, the author(s) did not use AI tools and the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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