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Sustainable Use of Sewage Sludge for Marigold (*Tagetes erecta* L.) Cultivation: Experimental and Predictive Modeling Studies on Heavy Metal Accumulation

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Abstract: The present study aimed to investigate the impact of sewage sludge (SS) amendment on the growth, yield, and biochemical attributes of the marigold (*Tagetes erecta* L. var. Pusa Basanti Gainda) crop. For this purpose, marigold flowers were cultivated using three different treatments of SS, i.e., 0% (control with no SS), 5%, and 10%. Multiple linear regression (MLR) modeling was performed to develop prediction models for the impact of soil properties on heavy metals uptake by marigold plants. The results showed that the growth, yield, and biochemical attributes of marigold plants significantly ($p < 0.05$) increased with an increase in SS dose from 0 to 10%. The most feasible SS treatment was found to be 10%, which achieved a maximum flower yield of 318.42 g/plant. On the other hand, the bioaccumulation factor (BAF) values (>1) showed that the marigold plant was capable of uptaking significant contents of six heavy metals in the order of Cd < Cr < Cu < Zn < Mn < Fe. The MLR-based predictive models were capable of precisely predicting the contents of most heavy metal uptake by marigold plants as indicated by the coefficient of determination (R^2 > 0.73), model efficiency (ME > 0.49), root mean square error (RMSE < 3.25), and analysis of variance (ANOVA; $p < 0.05$) results. Overall, this study presented a novel approach to floriculture by sustainable management of SS while reducing public health and environmental impacts.

Keywords: biochemical components; floriculture; mathematical modeling; multiple linear regression; waste management

1. Introduction

Sewage sludge (SS), a byproduct of the wastewater treatment process, is produced in huge quantities in urban areas [1]. At the global scale, nearly 75–100 million tons of SS is produced annually, which is estimated to hit 127.5 million tons by 2030 [2]. Being rich in organic and inorganic chemicals, SS can contaminate soil and water environments [3]. Therefore, SS management has appeared as one of the emerging environmental issues, especially for developing countries. Improper disposal of SS creates a wide range of environmental problems such as soil and water pollution, greenhouse gas emission, and the spread of diseases, among others [4]. This leads to the need for proper management of SS, as it is essential for protecting public health, the environment, and water resources.

In recent times, land application of SS has become an increasing trend, which includes the use of SS on agricultural lands for improving their fertility [5]. SS can also be managed via several other methods such as anaerobic digestion, composting, incineration, and disposal in landfills [6]. At sewage treatment facilities, sludge is pre-digested under anaerobic conditions to produce fuel gas (methane). The digested SS is dried and then used as a soil amendment for agricultural purposes [7]. Composting of SS stabilizes the organic and other nutrient elements, thereby making it easier to manage [8]. Although incineration removes organic debris, it can also release toxins such as dioxins, furans, pesticides, and flame retardants into the atmosphere [3,5]. Similarly, SS-landfill is also one of the widely used methods, but it may cause severe groundwater pollution, as indicated by recent studies [9].

Out of all the available methods, the controlled agricultural use of pre-digested SS is considered the most viable option to sustainably increase crop production and boost soil health [10]. Because of its high nutrient content, SS is an excellent fertilizer for a wide range of crops, including fruits, vegetables, and grains [11,12]. It also aids to increase soil fertility and structure by supplying several macronutrients such as nitrogen (N), phosphorous (P), and potassium (K), as well as micronutrients such as zinc (Zn), iron (Fe), and copper (Cu) [13]. Furthermore, SS can aid in the retention of moisture in the soil and the enhancement of the ability to absorb and retain nutrients. The European Union (EU) Council Directive regulates the agricultural reuse of SS, especially on its use for certain types of edible crops and land applications, thus ensuring the limit for potentially harmful contaminants [14]. However, SS reuse for the production of non-edible crops is one of the most viable options that ensure both soil and consumer safety [15]. Several non-edible crops such as flowers (marigold), energy (jatropha), building (bamboo), fiber (*Sesbania* spp.), pharmaceutical (borage), and biopolymer (rubber) can be cultivated using controlled doses of SS [16–18].

Floriculture, or simply flower farming, is the production of flowers and other ornamental plants used for decoration, temple offerings, cultural and ritual uses, and the extraction of essential oils, medicines, and foods. In India, numerous types of flowers, including marigold (*Tagetes* spp.), Hibiscus (*Hibiscus rosa-sinensis*), Pansy (*Viola tricolor*), Lotus (*Nelumbo nucifera*), Dahlia (*Dahlia pinnata*), Jasmine (*Jasminum officinale*), etc., are widely cultivated and have high market demand [19]. Among them, however, the marigold flower holds first rank in terms of the annual production of 1754 thousand metric tons [20], signifying its high market demand. Marigold (*Tagetes erecta* L.) flowers are mostly used for non-edible purposes; therefore, there is very less chance of heavy metals and other contaminants being consumed by human beings along with the flowers [21,22]. Thus, marigold cultivation can be an effective solution to SS management, and it can provide a productive and sustainable way to use this material. However, there has been little research on the effect of the SS amendment on heavy metal accumulation in marigold. Previous research has primarily examined the effects of SS amendment on crop yields and soil health. However, the effects of the SS amendment on heavy metal mobility and accumulation in marigold plants are largely unexplored.

Therefore, the present study aimed to investigate the effect of SS amendment on the growth, yield, and biochemical attributes of the marigold crop. Furthermore, multiple linear regression-based prediction models were developed in order to evaluate the influence of SS-amended soil properties on heavy metal uptake by marigold plants.

2. Materials and Methods

2.1. Material Collection

For the present study, SS was collected from a sewage treatment plant (STP) located in Saliyar, Roorkee, India (29°54′05.8″ N and 77°51′53.8″ E). This STP helps in treating the municipal wastewater generated from Roorkee city. SS samples were collected in 10 kg polyvinylchloride (PVC) plastic bags and transported to the experimental site at Kulheri village, Saharanpur, Uttar Pradesh, India (29°52′51.4″ N and 77°16′17.7″ E). Experiments were conducted in a polyhouse (5 × 3 × 10 m; width × height × length) constructed using polycarbonate sheets. Before the collected SS sample was used in experiments and analysis, the sample was sun-dried and stored at room temperature. On the other hand, arable soil (AS) of loamy texture was collected from agricultural land (up to 25 cm depth) near the experimental site. Moreover, authentic and healthy seeds of marigold (*Tagetes erecta* L. var. Pusa Basanti Gainda) were procured from Bhawani Seeds and Bio-Tech (Mathura, Uttar Pradesh, India).

2.2. Experimental Design

Marigold (*T. erecta*) cultivation experiments were conducted using different doses of SS mixed with AS, including 0% (absolute AS for control), 5%, and 10% (*w*/*w*) from November 2021 to March 2022. For this purpose, plastic pots of 30 kg capacity were taken and filled with 25 kg of an appropriate dose of AS mixed with SS. The marigold seeds were germinated on trays (Boxseat Ventures Pvt. Ltd., Delhi, India) in the same soil taken from plastic pots under dark conditions and frequent water spraying. Once seedlings reached 5 cm height, they were transferred to the pots after 20 days. A total of 30 pots were used for marigold cultivation (n = 10 replicated pots for each treatment), and one healthy seedling was carefully transplanted in each pot. The plants were raised carefully under greenhouse conditions for 140 days. The average temperature and humidity of the polyhouse were maintained at 25 °C and 55% with full-day sunlight. The flowering was observed from mid-February to mid-March 2022, and picking was performed once flowers reached maximum diameter (every fourth day) and marketable grade (according to size and color). The plants were irrigated equally with a borewell water supply using a hand sprayer while defective leaves and branches were immediately removed to avoid any pest or pathogen attack.

2.3. Chemical Analyses

In the current study, SS and AS collected from sampling sites were analyzed for selected physicochemical properties such as pH and electrical conductivity (EC: dS/m) using a microprocessor-based digital meter (ESICO 1611, Parwanoo, India), organic matter (OM: g/kg) using the Walkley and Black method [23], total nitrogen (TN: g/kg) using Kjeldahl's method [24], total phosphorus (TP: g/kg) using digestion–distillation and a spectrophotometer method (Cary 60, Agilent Technologies, Santa Clara, CA, USA), and total potassium (K: g/kg) using a flame-photometer method (1382, ESICO, Parwanoo, India) as previously adopted by Kumar et al. [25]. Moreover, AS and SS were also characterized for six heavy metals, including Cd, Cr, Cu, Fe, Mn, and Zn using atomic absorption spectroscopy (Analyst 800, PerkinElmer, Waltham, MA, USA). For this purpose, 1 g of oven-dried AS or SS sample was dissolved in double distilled water and then digested in a di-acid mixture having a 1:3 ratio of HNO_3 and $HClO_4$ on an electric hot plate (150° C for 1.5 h). For heavy metal analysis in the marigold plant, 1 g of oven-dried plant tissues, i.e., root, shoot, and flowers were separately taken and digested in the same way. Further, the sample was filtered through Whatman filter paper (number 41), and the total contents were adjusted to 50 mL by the addition of 3% HNO_3 solution for final determination using AAS (recovery percentage > 98%). Heavy metal standards of 0 to 50 mg/L were prepared and AAS was calibrated accordingly. The slit width of the instrument was adjusted to 0.5 nm, and an air/acetylene gas mixture was used for combustion [26]. The current of hollow cathode lamps for each metal was adjusted as per the manufacturer's guidelines and analytical results. All chemicals and reagents used in this study were of analytical grade and procured from Merck (India), and results were validated following standard operating procedures (SOPs) and qualitative assurance (QC) [27].

2.4. Plant and Biochemical Assays

Marigold plants were evaluated for selected growth, yield, and biochemical characteristics in order to understand the impact of SS application at different rates. Firstly, the selected growth and yield attributes such as plant height (cm), the number of branches, root length (cm), first bud formation (days), flowering period (days), flower stack length (cm), flower diameter (cm), flower yield (number per plant), total flower yield (g), and the average weight of flower (g) were manually estimated using calibrated scale and weighing balance (SP500, Samson, Mohali, India). Further, the total chlorophyll content of marigold plant leaves was determined following 80% acetone extraction and spectroscopy, as previously adopted by Shah et al. [28]. The contents of catalase (U/mL) and peroxidase (μmol/mg) were spectrophotometrically estimated at 240 nm [29] and 470 nm [30]. Similarly, the contents of β-carotene were determined by using acetone as an extraction agent and taking the absorbance at 450 nm, as previously described by de Carvalho et al. [31]. The contents of total phenols were estimated by using Folin and Ciocalteu's phenol reagents and taking absorbance at 725 nm while ascorbic acid was determined after extraction of methanolic contents followed by spectroscopic determination at 515 nm [32]. Finally, the contents of lutein were determined after extracting the contents using methanol and then analyzed using high-performance liquid chromatography (HPLC), as adopted by Šivel et al. [33].

2.5. Data Analysis and Software

In this study, the bioaccumulation factor (BAF) tool was used to study the heavy metal accumulation potential of marigold plants. BAF is a measure of how much a heavy metal accumulates in the vegetative parts of the marigold plant in comparison to the content in the soil [34]. BAF was calculated by dividing the heavy metal content (mg/kg) in the plant (HM_{plant}) by the heavy metal content (mg/kg) in the soil (HM_{soil}), as shown in Equation (1):

$$BAF = HM_{plant}/HM_{soil} \quad (1)$$

On the other hand, the multiple linear regression (MLR) method was used for developing the predictive models to understand the influence of selected soil properties on heavy metals uptake by the marigold plant parts (root, shoot, and flower). For this purpose, soil properties such as pH, OM, and total heavy metal content were used as independent variables. The following Equation (2) was used for the prediction of heavy metal uptake by the marigold plant as a dependent variable [35]:

$$y = \beta + \beta_1 \times pH_{soil} + \beta_2 \times OM_{soil} + \beta_3 \times HM_{soil} \quad (2)$$

where "y" is predicted heavy metal uptake by the marigold plant, "β" refers to the MLR intercept, while "β_1", "β_2", and "β_3" refer to interactive model coefficients for soil pH, OM, and HM, respectively. Moreover, the developed models were tested for goodness of fit and accuracy by using selected validation tools such as measured vs. predicted response values, coefficient of determination (R^2), Nash-Sutcliffe model efficiency (ME) coefficient, and root mean square error (RMSE). Thus, the following Equations (3)–(5) were used for the computation of R^2, ME, and RMSE:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(\widehat{y_{\text{obs}}} - \overline{y_{\text{pre}}})^2}{\sum_{i=1}^{n}(y_{\text{obs}} - \overline{y_{\text{mean}}})^2} \quad (3)$$

$$\mathrm{ME} = 1 - \frac{\sum_{i=1}^{n}(y_{\mathrm{obs}} - y_{\mathrm{pre}})^2}{\sum_{i=1}^{n}(y_{\mathrm{obs}} - \overline{y_{\mathrm{mean}}})^2} \quad (4)$$

$$\mathrm{RMSE} = \sqrt{\frac{\sum_{i=1}^{n}(y_{\mathrm{obs}} - y_{\mathrm{pre}})^2}{n}} \quad (5)$$

where "y_{obs}", "y_{pre}", and "y_{mean}" are experimental, predicted, and mean values of heavy metal content in different parts of marigold plants. All experiments were conducted in ten replicates, and data were presented as mean followed by standard deviation. The data were analyzed using an analysis of variance (ANOVA) test to compare experimental treatments (based on Tukey's) at a significance level of probability (p) < 0.05. The data analysis and statistical modeling were performed in Microsoft Excel (Version 2019, Microsoft Corp., Redmond, Washington, United States) and OriginPro (Version 2023, OriginLab Corp., Northampton, Massachusetts, United States) software packages.

3. Results and Discussion

3.1. Impact of Sewage Sludge on Soil Physicochemical Properties

The results of the physicochemical and heavy metal analyses of AS, SS, and experimental treatments (5% and 10%) are presented in Table 1. The analyses indicated that experimental treatments had a significantly ($p < 0.05$) lower pH (6.24–6.43) than AS. However, all studied soils showed a slightly acid-to-neutral pH, which can be suitable for marigold cultivation, since it grows perfectly on soils with a pH range of 6.2–6.5 [36]. This suggests that the experimental soils are the most suitable for the growth and development of this plant. ANOVA test outlined a significant increase ($p < 0.05$) in most parameters of experimental treatments in comparison with AS. In particular, the following physicochemical and heavy metal parameters were significantly higher ($p < 0.05$) in experimental treatments compared to the control (AS): EC (2.70–3.14 dS/m), OM (2.51–3.85 g/kg), TN (2.81–3.72 mg/L), TP (2.10–2.64 g/kg), TK (0.32–0.69 g/kg), Cu (6.50–9.23 mg/kg), Mn (10.56–12.31 mg/kg), and Zn (8.07–11.20 mg/kg). It is worth noting that the application of 10% SS to AS showed higher values in terms of the aforementioned parameters in comparison with 5% experimental soil. No significant difference ($p > 0.05$) in Fe was observed between AS and experimental treatments (17.40–21.60 mg/kg). However, 5% experimental soil treatment showed no significant difference ($p > 0.05$) with AS in terms of Cd and Cr, despite that these minerals were found significantly higher ($p < 0.05$) than AS when 10% SS was added to AS (0.45 and 5.38 mg/kg, respectively).

Table 1. Average characteristics of sewage sludge, experimental, and control arable soils used for marigold cultivation.

Properties	Arable Soil	Sewage Sludge	Experimental Treatments	
			5%	10%
pH	6.74 ± 0.04 ^{d}	5.98 ± 0.07 ^{a}	6.43 ± 0.07 ^{c}	6.24 ± 0.05 ^{b}
Electrical conductivity (EC: dS/m)	2.40 ± 0.03 ^{a}	6.30 ± 0.12 ^{d}	2.70 ± 0.06 ^{b}	3.14 ± 0.08 ^{c}
Organic matter (OM: g/kg)	1.39 ± 0.04 ^{a}	25.04 ± 2.80 ^{c}	2.51 ± 0.11 ^{b}	3.85 ± 0.14 ^{bc}
Total nitrogen (TN: g/kg)	1.70 ± 0.02 ^{a}	20.99 ± 1.58 ^{d}	2.81 ± 0.03 ^{b}	3.72 ± 0.07 ^{c}
Total phosphorus (TP: g/kg)	1.26 ± 0.05 ^{a}	14.75 ± 0.94 ^{d}	2.10 ± 0.05 ^{b}	2.64 ± 0.10 ^{c}
Total potassium (TK: g/kg)	0.10 ± 0.02 ^{a}	5.09 ± 0.17 ^{d}	0.32 ± 0.04 ^{b}	0.69 ± 0.09 ^{c}
Cadmium (Cd: mg/kg)	0.27 ± 0.05 ^{a}	1.98 ± 0.10 ^{c}	0.38 ± 0.06 ^{a}	0.45 ± 0.04 ^{b}
Chromium (Cr: mg/kg)	3.64 ± 0.20 ^{a}	14.62 ± 3.02 ^{c}	4.26 ± 0.41 ^{a}	5.38 ± 0.27 ^{b}
Copper (Cu: mg/kg)	4.10 ± 0.28 ^{a}	49.32 ± 5.90 ^{d}	6.50 ± 0.24 ^{b}	9.23 ± 0.56 ^{c}
Iron (Fe: mg/kg)	17.40 ± 2.46 ^{a}	41.60 ± 4.08 ^{b}	19.42 ± 1.87 ^{a}	21.60 ± 2.01 ^{a}
Manganese (Mn: mg/kg)	9.06 ± 0.54 ^{a}	32.03 ± 5.62 ^{d}	10.56 ± 0.34 ^{b}	12.31 ± 1.14 ^{c}
Zinc (Zn: mg/kg)	3.80 ± 0.40 ^{a}	84.20 ± 8.28 ^{d}	8.07 ± 0.16 ^{b}	11.20 ± 0.48 ^{c}

Values are mean followed by the standard deviation of ten replicates; $^{a–d}$: the same letters indicate no significant difference between treatment groups at $p < 0.05$.

An increase in the soil EC enhances the solubility of nutrients [31], thus their higher availability for the growth of cultivated marigold. The increased OM content in experimental soils would result in improved soil structure and permeability [37]. Although crucial for crop growth development, high TN, TP, and TK levels can lead to the dominance of invasive alien plants and reduced soil microbial population [38]. The latter is responsible for the promotion of crop growth and development via several simple and complex mechanisms [26,39]. Soil heavy metals were in the following decreasing order: Fe > Mn > Cu > Zn > Cr > Cd. Despite being essential microelements, higher levels of these metals can bring cytotoxic effects on soils, and phytotoxicity in growing crops, which is associated with stunt growth and chlorosis [40]. However, the current values are low and do not show any potential risk to soil health and quality, and further on the cultivated marigold.

3.2. Impact of Sewage Sludge on Growth and Yield of Marigold

Table 2 shows the impact of SS amendment on the growth, yield, and flower characteristics of cultivated marigold. Results showed that 5% and 10% SS experimental treatments significantly ($p < 0.05$) improved the growth and performance of marigold. More specifically, plant height (52.40 cm), number of branches (11.06), and root length (21.48 cm) were the highest when AS was amended with 10% SS, followed by 5% experimental soil compared to the control. Several studies reported the potential and safe use of SS in agricultural activities yielding promising yields [25,41–43]. Besides this, it is considered safer than chemical and other organic fertilizers. In order to ensure that SS is safe for use as fertilizer, it undergoes a rigorous treatment process such as screening, settling, disinfection, digestion, and drying that help remove pathogens, heavy metals, and other contaminants. Additionally, SS has a slow-release nutrient profile, which means that the nutrients are released slowly over time, reducing the risk of over-fertilization and nutrient leaching [25]. Bi et al. [44] amended greenhouse-cultivated French marigold with broiler chicken litter-based organic fertilizers. They observed increased plant growth parameters including root rate and demonstrated the positive impact of organic fertilizers on marigold cultivated under greenhouse conditions. The increased root length may be due to increased soil porosity as a result of organic matter improvement [37]. This corroborates with the findings outlined in Table 1 in this regard. The current study proves the potential role of SS in the sustainable production of marigold.

Table 2. Impact of sewage sludge amendment on growth, yield, and flower characteristics of cultivated marigold.

Properties	Arable Soil	Sewage Sludge Treatment	
		5%	10%
Plant height (cm)	39.08 ± 2.72 ^{a}	48.11 ± 1.24 ^{b}	52.40 ± 3.57 ^{b}
Number of branches (no.)	7.13 ± 0.47 ^{a}	10.41 ± 0.37 ^{b}	11.06 ± 1.02 ^{b}
Root length (cm)	17.30 ± 1.13 ^{a}	20.09 ± 0.95 ^{b}	21.48 ± 0.69 ^{b}
First bud formation (days)	130.25 ± 4.75 ^{c}	119.80 ± 2.20 ^{b}	110.38 ± 3.72 ^{a}
Flowering period (days)	45.50 ± 2.50 ^{a}	57.20 ± 1.80 ^{b}	62.46 ± 2.54 ^{c}
Flower stack (cm)	8.03 ± 0.04 ^{a}	8.93 ± 0.10 ^{b}	9.15 ± 0.15 ^{c}
Flower diameter (cm)	5.51 ± 0.09 ^{a}	6.02 ± 0.05 ^{b}	6.16 ± 0.07 ^{c}
Flower yield (no. per plant)	24.08 ± 0.10 ^{a}	26.59 ± 0.24 ^{b}	26.35 ± 0.20 ^{b}
Flower yield (g per plant)	255.28 ± 5.15 ^{a}	306.90 ± 1.86 ^{b}	318.42 ± 3.09 ^{c}
Average weight of flower (g)	10.60 ± 0.10 ^{a}	11.54 ± 0.09 ^{b}	12.08 ± 0.16 ^{c}

Values are mean followed by the standard deviation of ten replicates; $^{a–c}$: the same letters indicate no significant difference between treatment groups at $p < 0.05$.

Several factors have been reported to control flowering initiation, which include irradiance, photoperiod, stresses, temperature, and the extra levels of nutrients in the soil [45–47]. Our findings outlined a significantly ($p < 0.05$) shorter time to first bud formation in experimental treatments (119.80 and 110.38 days), and a significantly ($p < 0.05$) extended flowering period was observed (57.20 and 62.46 days) compared to the control. The earlier budding can be a result of high metabolite uptake by the shoots and roots of marigold. Such observation was previously denoted on sunflowers amended with organic fertilizers [48], whereas the extended flowering period may be a result of increased cytokinin production [49]. Flower stack (9.15 cm), flower diameter (6.16 cm), flower yield (318.42 g/plant), and average flower weight (12.08 g) were highest in the 10% experimental treatment. These flower parameters were significantly ($p < 0.05$) higher in experimental treatments compared to the control (10% SS > 5% SS > AS). Flower yield was the highest in 5% experimental treatment (26.59 flowers/plant), followed by 10% treatment (26.35 flowers/plant) and AS (24.08 flowers/plant). Increased flower diameter can be a result of increased salicylic acid production by the marigold [50]. Such observation was also denoted on rose flowers [51]. Increased yield and number of flowers per plant were also reported on French marigold plants when the latter was amended with broiler chicken litter-based organic fertilizers [44]. Therefore, the findings of the present study suggest SS is a promising organic fertilizer for improving the growth and yield parameters of cultivated marigold.

3.3. Impact of Sewage Sludge on Biochemical Characteristics of Marigold

The biochemical composition of marigold grown on control and SS-amended soils is presented in Table 3. Total chlorophyll content and peroxidase activity were significantly ($p < 0.05$) higher in the leaves of experimental treatments (2.47–2.52 mg/g; 4.87–6.20 μmol/mg) compared to those of the control. Peralta-Sánchez et al. [52] earlier reported a total chlorophyll content of 0.75 mg/g in the leaves of marigold. Such differences can be explained by strain type, soil composition, and environmental factors. The use of organic fertilization showed its influence on the increase in total chlorophyll content of various crops [26,35], whereas the peroxidase activity in marigold leaves was reported to be 85–90 U/min/g in the study of Tian et al. [53]. These authors outlined the effect of cultivar variation on the antioxidant activity found in marigold leaves. In the present study, the catalase activity in marigold leaves was significantly ($p < 0.05$) lower in 10% SS treatment compared to the control (2.15 and 2.90 U/mL, respectively), whereas 5% treatment did not show such a significant difference (2.75 U/mL). Tian et al. [53] mentioned a catalase activity of 11.5–12.5 U/min/g in many marigold cultivars. Marigold flowers did not contain any chlorophyll content nor possessed catalase or peroxidase activities in all treatments. It is well known that the presence of chlorophyll content in flowers is a very rare phenomenon in developed stages; only low contents can be found in early development stages [54]. However, the lack of catalase and peroxidase activities in the marigold flowers of the present study can result in the non-scavenging of H_2O_2 [55].

Table 3. Impact of sewage sludge amendment on biochemical characteristics of flower and leaves of cultivated marigold.

Properties	Plant Part	Arable Soil	Sewage Sludge Treatments	
			5%	10%
Total chlorophyll (mg/g)	Leaves	2.30 ± 0.05 [a]	2.47 ± 0.02 [b]	2.52 ± 0.06 [b]
	Flowers	*na*	*na*	*na*
Catalase (U/mL)	Leaves	2.90 ± 0.10 [a]	2.75 ± 0.07 [a]	2.15 ± 0.03[b]
	Flowers	*na*	*na*	*na*
Peroxidase (μmol/mg)	Leaves	3.10 ± 0.06 [a]	4.87 ± 0.05 [b]	6.20 ± 0.18 [c]
	Flowers	*na*	*na*	*na*
β-carotene (μg/g)	Leaves	15.93 ± 0.30 [a]	17.36 ± 1.02 [a]	19.09 ± 0.95 [b]
	Flowers	10.08 ± 0.07 [a]	10.51 ± 0.10 [b]	11.56 ± 0.09 [c]
Total phenols (mg/g)	Leaves	12.10 ± 0.12 [a]	15.44 ± 0.28 [b]	17.07 ± 0.50 [c]
	Flowers	33.48 ± 2.09 [a]	48.30 ± 4.58 [b]	51.03 ± 3.75 [b]
Ascorbic acid (mg/g)	Leaves	1.60 ± 0.05 [a]	1.73 ± 0.04 [b]	1.96 ± 0.11 [bc]
	Flowers	0.52 ± 0.02 [a]	0.67 ± 0.05 [b]	0.74 ± 0.08 [b]
Flavonoids (mg/g)	Leaves	55.40 ± 3.84 [a]	62.90 ± 1.90 [b]	64.11 ± 2.75 [b]
	Flowers	36.27 ± 1.51 [a]	41.02 ± 3.02 [b]	45.20 ± 2.48 [b]
Lutein (mg/g)	Leaves	47.10 ± 2.07 [a]	51.75 ± 2.72 [b]	53.92 ± 1.01 [b]
	Flowers	70.49 ± 1.43 [a]	78.38 ± 2.54 [b]	84.66 ± 3.13 [c]

Values are mean followed by the standard deviation of ten replicates; [a–c]: the same letters indicate no significant difference between treatment groups at $p < 0.05$; *na*: not applicable.

Our results also showed that β-carotene, ascorbic acid, and flavonoid contents were higher in the leaves than in flowers, whereas the contrary was observed regarding total phenols and lutein contents. β-carotene content was significantly ($p < 0.05$) higher in 10% treatment in comparison with the control (19.09 and 15.93 μg/g, respectively). However, no significant difference was observed between the 5% treatment (17.36 μg/g) and the control. On the other hand, marigold flowers of experimental treatments contained significantly ($p < 0.05$) higher β-carotene content (10.51–11.56 μg/g) compared to the control. Such increases can be explained by the increased gene expression and thus increased yellowish and orangish color of the flowers [56]. The present study reported total phenols contents of 12.10–17.07 mg/g, ascorbic acid contents of 1.60–1.96 mg/g, flavonoid contents of 55.40–64.11 mg/g, and lutein contents of 47.10–53.92 mg/g in marigold leaves, which were significantly higher ($p < 0.05$) in experimental treatments than in the control (10% > 5% > control). Similarly, marigold flowers enclosed total phenols contents of 33.48–51.03 mg/g, ascorbic acid contents of 0.52–0.74 mg/g, flavonoid contents of 36.27–45.20 mg/g, and lutein contents of 70.49–84.66 mg/g, which were significantly higher ($p < 0.05$) in experimental treatments than in the control (10% > 5% > control).

The increased total phenols in marigold leaves and flowers correspond to increased bioactivity and thus better antioxidant and antibacterial activities [57]. It was also reported that increased flavonoid content in French marigold resulted in improved cytoprotective activity [58]. Peralta-Sánchez et al. [52] mentioned total flavonoid contents of 1.1 and 3.7 mg/g DW in the leaves and flowers of Mexican marigold. The increase in ascorbic acid in marigold leaves improves the regulation of the redox state of photosynthetic electron carriers, thereby better photosynthesis mechanism [59]. Krzymińska et al. [60] denoted a dependence of phenolic compounds, ascorbic acid, and flavonoids on the growing substrate of marigold plants. Recent reports depicted the role of lutein on the yellow color intensity of marigold flowers [61]. Furthermore, this component plays an important role in the triggering of ROS generation [62]; thus, SS amendment to marigold plants is very promising.

3.4. Impact of Sewage Sludge on Heavy Metal Accumulation in Marigold

Results in Table 4 outlined a gradual decrease in heavy metal accumulation from the lower to upper parts of marigold plants (root > shoot > flower) in all treatments. This is a natural phenomenon in which root vacuoles tend to reduce the translocation of such potentially toxic elements to shoots [63]. As a result, the negative impacts on shoot cells, chloroplasts, and mitochondria are diminished and associated with a lower oxidative stress heaviness [64]. The current study reported Cd contents of 0.21–0.72 mg/kg, Cr contents of 0.90–1.52 mg/kg, Cu contents of 3.50–8.40 mg/kg, Fe contents of 12.40–34.02 mg/kg, Mn contents of 5.71–14.09 mg/kg, and Zn contents of 7.62–11.83 mg/kg in significantly higher marigold roots ($p < 0.05$) in experimental treatments than in the control (10% > 5% > control). Recently, Biswal et al. [65] revealed high translocation factors of Ni and Cd from polluted sites to the roots of two marigold species, namely, *T. patula* and *T. erecta*. These authors also found a correlation between increased flower yield and heavy metal bioaccumulation in marigold. Such findings corroborate our findings, where increased heavy metals in experimental treatments resulted in higher yields than in the control. Therefore, marigold can be considered a good bioremediating agent for soils polluted with SS, while the latter can play an interesting role in the improvement of marigold physicochemical characteristics. Cd and Cr contents were significantly ($p < 0.05$) more accumulated in marigold shoots of 10% treatment than in the control (0.19 and 1.10 mg/kg, respectively). However, marigold shoots bioaccumulated Cu contents of 2.17–5.73 mg/kg, Fe contents of 17.37–22.30 mg/kg, Mn contents of 4.02–10.67 mg/kg, and Zn contents of 5.27–9.35 mg/kg, which were significantly higher ($p < 0.05$) in experimental treatments than in the control (10% > 5% > control). Madanan et al. [66] reported higher bioaccumulation of Cd in roots and shoots of marigold than Zn. Our findings showed an irregular pattern in both the control and experimental treatments. The flowers harvested from experimental treatments showed no significant difference in Cd content in comparison with the control (0.01–0.02 mg/kg), whereas the flowers of 10% treatment bioaccumulated significantly ($p < 0.05$) had a higher Cr content than the control ones (0.22 and 0.16 mg/kg, respectively). Moreover, marigold flowers bioaccumulated Cu contents of 1.88–2.50 mg/kg, Fe contents of 3.53–5.10 mg/kg, Mn contents of 1.06–1.40 mg/kg, and Zn contents of 2.50–4.21 mg/kg, which were significantly higher ($p < 0.05$) in experimental treatments than in the control (10% > 5% > control). Such increases in heavy metals can ruin the whole bioremediation process if marigold flowers are hazardously disposed of into the environment after being used in temples for religious purposes. Therefore, the recycling of marigold flowers grown on SS-treated soils in various biological and chemical processes is highly required.

Table 4. Impact of sewage sludge amendment on heavy metal accumulation in different plant parts of cultivated marigold.

Heavy Metal	Plant Part	Arable Soil	Sewage Sludge Treatments	
			5%	10%
Cd	Root	0.21 ± 0.02 [a]	0.48 ± 0.05 [b]	0.72 ± 0.07 [c]
	Shoot	0.12 ± 0.03 [a]	0.16 ± 0.02 [a]	0.19 ± 0.05 [b]
	Flowers	0.01 ± 0.00 [a]	0.02 ± 0.01 [a]	0.02 ± 0.01 [a]
Cr	Root	0.90 ± 0.07 [a]	1.37 ± 0.10 [b]	1.52 ± 0.19 [bc]
	Shoot	0.74 ± 0.05 [a]	0.83 ± 0.04 [a]	1.10 ± 0.08 [b]
	Flowers	0.16 ± 0.02 [a]	0.19 ± 0.03 [ab]	0.22 ± 0.02 [b]
Cu	Root	3.50 ± 0.09 [a]	6.94 ± 0.25 [b]	8.40 ± 0.71 [c]
	Shoot	2.17 ± 0.04 [a]	4.63 ± 0.16 [b]	5.73 ± 0.32 [c]
	Flowers	1.88 ± 0.11 [a]	2.10 ± 0.08 [b]	2.50 ± 0.29 [c]
Fe	Root	12.40 ± 1.82 [a]	26.62 ± 3.01 [b]	34.02 ± 4.35 [c]
	Shoot	17.37 ± 0.60 [a]	20.10 ± 1.25 [ab]	22.30 ± 0.94 [b]
	Flowers	3.53 ± 0.20 [a]	4.86 ± 0.52 [b]	5.10 ± 0.17 [c]
Mn	Root	5.71 ± 0.19 [a]	12.18 ± 2.40 [b]	14.09 ± 1.64 [bc]
	Shoot	4.02 ± 0.08[a]	8.49 ± 0.65 [b]	10.67 ± 1.28 [bc]
	Flowers	1.06 ± 0.04[a]	1.34 ± 0.07 [b]	1.40 ± 0.05 [c]
Zn	Root	7.62 ± 0.30[a]	9.91 ± 0.55 [b]	11.83 ± 1.02 [bc]
	Shoot	5.27 ± 0.12[a]	8.02 ± 0.19 [b]	9.35 ± 0.24 [c]
	Flowers	2.50 ± 0.09[a]	3.98 ± 0.07 [b]	4.21 ± 0.11 [bc]

Values are mean followed by the standard deviation of ten replicates; [a–c]: the same letters indicate no significant difference between treatment groups at $p < 0.05$.

The bioaccumulation factor (BAF) evaluation is an important step to detect which heavy metal can be harmful or cause deleterious impacts on the grown crops [34]. In this study, Cd bioaccumulation was safe (BAF < 1.00) except for 5% and 10% treatments' roots (1.26 and 1.60), whereas Cr bioaccumulation was safe (BAF < 1.00) in all treatments and plant parts (Figure 1). Cu bioaccumulation was safe (BAF < 1.00) except for 5% treatment's roots (BAF: 1.05). Fe bioaccumulation showed to be highest in 5% of treatment shoots (BAF: 1.92). Mn bioaccumulation was generally moderate (BAF < 1.00), except in experimental treatments' roots (BAF: 1.15). However, the highest threat was attributed to Zn, which showed to be heavily bioaccumulated in all treatments' roots (1.06 < 1.23 < 2.01). In this context, Madanan et al. [66] reported an unsafe BAF (BAF > 1) for Cd of marigold roots grown on lateritic-polluted soils. These findings corroborate our obtained results from the experimental treatments. Further, the same authors also denoted high BAF (BAF > 1) for Zn of the same species' shoots. However, such an observation was not found in the experimental treatments of the present study.

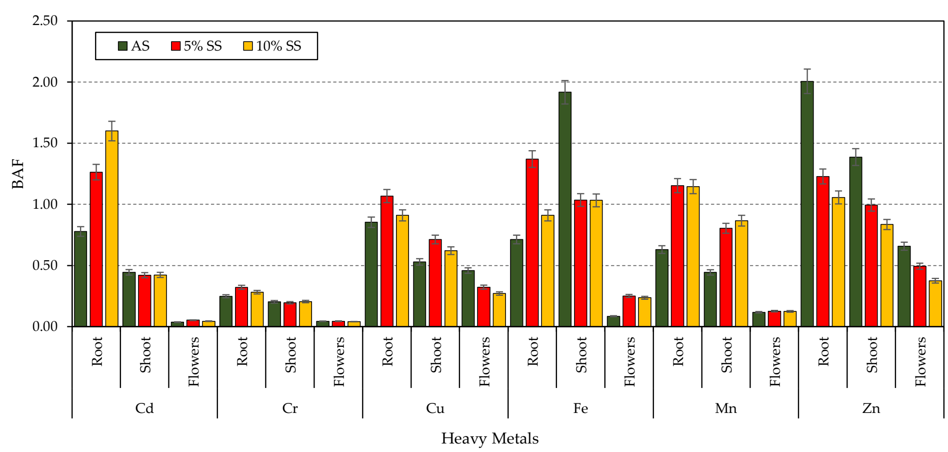

Figure 1. Bioaccumulation factor (BAF) of six heavy metals in different plant parts of marigold cultivated in different treatments (AS: arable soil as control, SS: sewage sludge).

3.5. Prediction Models for Heavy Metal Uptake by Marigold

Table 5 shows the results of prediction models for the uptake of heavy metal by different vegetative parts of marigold plants cultivated on SS-amended soils. In particular, the models were able to accurately predict the uptake amount of selected heavy metals, i.e., Cd, Cr, Cu, Fe, Mn, and Zn. As explained from the MLR model, it was observed that pH showed a negative association with the uptake of all heavy metals except for Cd (shoot and flowers), Cr (shoot), Cu (flower), and Mn (root). Additionally, OM showed a negative influence except for Cd (root and shoot), Cr (shoot), Fe (shoot), and Mn (root). However, the total heavy metal content showed a positive influence on their uptake by marigold plants except for Cr (shoot), and Mn (flowers). Cu accumulation in marigold flowers may be related to the presence of Cu transporters in their cells. In addition, the concentration of Cu in the plant may vary depending on the stage of growth and development. The amount of heavy metals that plants absorb depends significantly on the pH of the soil. Low soil pH enhances the availability of heavy metals and facilitates their uptake by plants. Similarly, the amount of OM in the soil can affect how well plants absorb heavy metals. Heavy metals can attach to OM, which makes them more accessible for plants to absorb [36]. The ANOVA test explained the suitability of models since *p*-values remain within the significance level of < 0.05 except for the developed model for Cd prediction in the flower region. Notwithstanding, the models showed relatively high coefficient of determination (R^2) values ranging from 0.73 to 0.99, which were acceptable. With root mean square error (RMSE) values of 0.01–3.25, the models were able to correctly forecast the amount of heavy metal uptake by the marigold plant. This was further supported by the range of predicted response values (y_{min} and y_{max}).

Table 5. Prediction models for uptake of heavy metal by different vegetative parts of marigold cultivated on sewage sludge amended soils.

Heavy Metals	Plant Parts	Model Equation	y_{min}	y_{max}	R^2	ANOVA		ME	RMSE
						F-Value	*p*-Value		
Cd	Root	$y = 0.40 - 0.08\ pH_{soil} + 0.13\ OM_{soil} + 0.73\ Cd_{soil}$	0.17	0.76	0.99	297.64	<0.01	0.98	0.04
	Shoot	$y = -0.76 + 0.10\ pH_{soil} + 0.01\ OM_{soil} + 0.46\ Cd_{soil}$	0.09	0.21	0.88	12.55	<0.01	0.75	0.12
	Flowers	$y = -0.09 + 0.01\ pH_{soil} - 0.01\ OM_{soil} + 0.12\ Cd_{soil}$	0.01	0.03	0.73	4.66	0.06	0.49	0.01
Cr	Root	$y = 13.62 - 2.18\ pH_{soil} - 0.80\ OM_{soil} + 0.87\ Cr_{soil}$	0.89	1.58	0.84	9.04	0.04	0.98	0.06
	Shoot	$y = -9.04 + 1.47\ pH_{soil} + 0.66\ OM_{soil} - 0.30\ Cr_{soil}$	0.71	1.18	0.99	236.72	<0.01	0.88	0.08
	Flowers	$y = 1.59 - 0.27\ pH_{soil} - 0.14\ OM_{soil} + 0.16\ Cr_{soil}$	0.14	0.23	0.95	36.95	<0.01	0.72	0.01
Cu	Root	$y = 39.40 - 6.00\ pH_{soil} - 5.65\ OM_{soil} + 2.07\ Cu_{soil}$	3.27	9.20	0.96	46.87	<0.01	0.94	0.80
	Shoot	$y = 29.48 - 4.45\ pH_{soil} - 3.06\ OM_{soil} + 1.73\ Cu_{soil}$	2.10	6.16	0.06	53.32	<0.01	0.97	0.43
	Flowers	$y = -5.22 + 0.86\ pH_{soil} - 0.69\ OM_{soil} + 0.53\ Cu_{soil}$	1.71	2.74	0.98	112.21	<0.01	0.55	0.24
Fe	Root	$y = 177.89 - 29.78\ pH_{soil} - 0.87\ OM_{soil} + 2.12\ Fe_{soil}$	8.93	37.27	0.96	53.08	<0.01	0.90	3.25
	Shoot	$y = 16.93 - 1.17\ pH_{soil} + 1.08\ OM_{soil} + 0.39\ Fe_{soil}$	16.50	23.31	0.97	71.26	<0.01	0.85	1.01
	Flowers	$y = 27.93 - 4.14\ pH_{soil} - 0.64\ OM_{soil} + 0.26\ Fe_{soil}$	3.16	5.40	0.88	13.06	<0.01	0.81	0.30
Mn	Root	$y = 37.16 - 7.13\ pH_{soil} - 0.72\ OM_{soil} + 2.02\ Mn_{soil}$	5.61	16.60	0.85	0.68	<0.01	0.82	2.51
	Shoot	$y = 48.54 - 8.50\ pH_{soil} - 1.16\ OM_{soil} + 1.61\ Mn_{soil}$	3.76	12.16	0.96	43.56	<0.01	0.89	1.49
	Flowers	$y = 3.96 - 0.54\ pH_{soil} - 0.10\ OM_{soil} - 0.10\ Mn_{soil}$	1.05	1.49	0.86	11.08	<0.01	0.85	0.09
Zn	Root	$y = -34.96 + 5.77\ pH_{soil} + 0.27\ OM_{soil} + 0.86\ Zn_{soil}$	7.04	12.60	0.98	147.54	<0.01	0.89	0.77
	Shoot	$y = 5.57 - 0.32\ pH_{soil} - 0.99\ OM_{soil} + 0.86\ Zn_{soil}$	5.00	9.63	0.99	301.78	<0.01	0.98	0.28
	Flowers	$y = 3.21 - 0.19\ pH_{soil} - 1.42\ OM_{soil} + 0.69\ Zn_{soil}$	2.52	4.35	0.98	120.04	<0.01	0.98	0.14

y: predicted heavy metal content (mg/kg); R^2: coefficient of determination; ME: model efficiency; RMSE: root mean square error.

MLR is a widely accepted approach for monitoring heavy metal contamination in both edible and non-edible crops. A recent study by Zhou et al. [67] constructed prediction models for the uptake of Cd by wheat-rice rotation crop system. The authors reported that the adopted approach was used for precise prediction of Cd contents in root, stem, and leaf, while pH and OM showed significant association with the uptake process. Similarly, Yu et al. [68] utilized the MLR method for heavy metal uptake by wheat crops fertigated with SS. The authors found that the developed models were efficient to predict the contents of six heavy metals, including Cd, Cu, Pb, Zn, Cr, and Ni while taking pH and OM as independent variables. Recently, Eid et al. [35] utilized MLR equations for uptake prediction of nine heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) by forage sorghum (*Sorghum bicolor* L.) crop. Their results showed the metal tissues in plant parts exhibited a negative correlation with soil pH, whereas OM, EC, and plant metal contents were all found to be positively correlated. The findings reported in the above studies are in line with those obtained in the present study; thus, the developed equations can be used for the prediction of heavy metal content absorbed by different parts of the marigold plant (root, shoot, and flower).

4. Conclusions

The results of this study concluded that SS can be effectively utilized for marigold cultivation. The maximum values for the growth, yield, and biochemical constituents of marigold were reported using 10% SS treatment. Moreover, the heavy metal analysis showed that the marigold accumulated the maximum contents of selected heavy metals in root parts followed by shoot and flowers. The bioaccumulation factor values >1 indicated that the marigold plant can be a useful candidate for the reclamation of SS-treated soils. Moreover, the multiple linear regression-based prediction models showed good fitness and high accuracy, as indicated by the validation results. Therefore, the most feasible improvement in the productivity of the marigold crop was reported using a 10% SS application, which suggests its sustainable suitability for floriculture. Additional research is required to better understand the mechanisms involved in the heavy metal uptake process by marigold plants as well as to test the suitability of SS for the cultivation of other flower species.

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