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Utilization of Municipal Wastewater Sludge for Enhancing Growth and Dye Yield in Pot Marigold Plants

Anahita Rashtian*, Mohammad Mosavi Hasanabad

Department of Range and Watershed Management, Faculty of Natural Resources, Yazd University, Yazd, Iran

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ABSTRACT

The appropriate disposal of municipal wastewater sludge as an organic fertilizer, alongside the production of wastewater effluents, has posed significant technical and environmental challenges in wastewater treatment plant management worldwide, including Iran. Contamination with heavy metals and pathogenic microorganisms has been a major limiting factor in the use of sludge as an organic fertilizer, rich in nutrients, for improving soil quality in agricultural fields. An experiment was conducted on the pot marigold Parpar cultivar, using a randomized complete block design with three replications. The sludge treatments were utilized at three levels, including control (no sludge), 10, and 20 $t.ha^{-1}$. The sludge was applied to field plots in a greenhouse located in Taft County, Yazd Province, Iran. Eleven plant performance indices were measured, and wool yarn was dyed with petal extracts. The hunter parameters in the CIE Lab system were used to assess lightfastness and washfastness. The results revealed that sludge application (20 t ha^{-1}) significantly enhanced six performance indices, including plant height (61%), the number of flowers per plant (58%), fresh weight of plant(42%), flower yield (74%), 1000-seeds weight (47%), and the number of capitula.m^{-2} (84%). Additionally, sludge application at a rate of "10 $t.ha^{-1}$" increased the peak absorption of the dye. The color of plants treated with 10 $t.ha^{-1}$ of sludge exhibited significantly higher washfastness, while those treated with 20 $t.ha^{-1}$ of sludge exhibited higher lightfastness compared to the control treatment.

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1. Introduction

Urban wastewater sludge was once considered a pollution source, whereas it is now regarded as a rich source of essential plant nutrients (Haritash *et al.*, 2015). This is significant from two perspectives: firstly, it addresses pollution control by preventing its release into rivers and preserving the environment. Secondly, the nutrients present in sludge are highly beneficial for plants. However, the presence of micronutrients and toxic elements such as lead, cadmium, mercury, and nickel limits the indiscriminate use of sludge in agricultural lands (Zeng *et al*., 2016). Previous studies have demonstrated that wastewater sludge has a high affinity for heavy metal sorption. Furthermore, the complexation of heavy metals by organic matter functional groups and their precipitation with phosphorus compounds in sludge can significantly reduce the bioavailability of heavy metals in plants (Lyu *et al.*, 2022). Plant cultivation with sludge fertilization has shown that non-essential elements such as lead, zinc, and cadmium accumulate in plants, but sludge can increase plant growth indices such as plant height and fresh and dry weight (Marjovvi and Mashayekhi, 2018). Reports have concluded that excessive sludge application leads to a significant accumulation of heavy metals in the soil, increasing the concentrations of cadmium (Cd), lead (Pb), mercury (Hg), and nickel (Ni) up to 100 times their baseline levels (Zeng *et al.*, 2016) also demonstrated that the active ingredients of Pot marigold are produced and stored in the flowers (Egeli, 2022). The most important of these include water-soluble flavonoids, carotenoids, essential oils, mucilage, and vitamin E. Pot marigold flowers also contain calendulin, saponin, cholesterol,

* Corresponding author.
E-mail address: arashtian@yazd.ac.ir

sterols, and steroidal glycoside lanolin (Egeli, 2022). The availability of essential elements in the soil can affect the intensity, hue, and overall color of plants. Research predominantly concentrates on elucidating the effects of varying soil conditions and nutrient levels on pigment production, as well as the mechanisms by which plants adapt to these changes (Lyu *et al*., 2022). Plant color changes in response to different soil conditions. Accordingly, aluminum and phosphorus ions can change plant color from blue to red and even affect color stability in plants (Kodama *et al*., 2016). On the other hand, micronutrients such as iron (Fe), zinc (Zn), and boron (B) are essential for plant growth and significantly affect plant color and quality. They extend beyond basic nutrition and influence various essential physiological processes for optimal growth (Lyu *et al*., 2022).

The wastewater application did not result in a significant difference in the quality traits of saffron, particularly in the content of picrocrocin, a key compound responsible for saffron's color (Choopan et *al*., 2021). In another study, the interaction between wastewater sludge and quinoa revealed that the sludge application significantly increased plant growth indices, including dry weight and photosynthetic pigments (Afzalinejad *et al*., 2021). Additionally, the impact of soil salinity on the pigments of the dye plant madder (*Rubia tinctorum*) demonstrated that as salinity increased, lightness (L^*) decreased, while yellowness and blueness increased (Mirmohammady Maibody *et al*., 2019). Potassium nitrate soil treatment produced the highest dry matter yield, while the best A^* index in yellow flowers and B^* index in orange flowers were found in soil treatments with humic acid (Nourolahi *et al*., 2019). The effect of cadmium on the pigments of mint (*Mentha aquatica*), eryngo (*Eryngium caucasicum*), and *Froriepia subpinnata* showed that phenolic and flavonoid pigments increased under cadmium stress (Hasanpour *et al*., 2019), whereas the mountain melic (*Melica nutans* L.) exhibited higher carotenoid pigments in low-fertility soils (Zielewicz *et al*., 2020).

Pot marigold *(Calendula officinalis* L.*)*, from the Asteraceae family, is a highly important plant with a long history of both ornamental and medicinal uses, with numerous therapeutic properties (Egeli, 2022). This plant contains approximately 15-20% oil, with 45-60% of that being calendic acid (Egeli, 2022). In addition to its culinary applications as a flavoring and coloring agent, it contains active compounds used in the production of paints, industrial nylon, and the pharmaceutical industry for making various creams and lotions (Silva *et al*., 2021). The yellow pigments lutein and zeaxanthin, both xanthophylls found in *C. officinalis*, exhibit strong antioxidant properties (Shahane *et al*., 2023).

Previous research on *C. officinalis* has been limited in exploring the effects of nutrition and soil on its color and dyeing properties. Given that plant nutrition and soil elements influence both plant chemical composition and pigment content (Nourolahi *et al*., 2019; Zielewicz *et al*., 2020), this study investigated the impact of using wastewater sludge as a fertilizer to enhance the growth and production of *C. officinalis*. Additionally, the study examined the effect of sludge on wool dyeing properties and aimed to determine the optimal application rate for cultivating this plant.

2. Materials and methods

This experiment was conducted in a private greenhouse in Cham village, Taft City, Iran over the 2020-2021 agricultural year in autumn. The experiment followed a randomized complete block design (RCBD) with three replications. Besides, three treatments of primary dewatered municipal sewage sludge were considered, including, control (no sludge), 20 $t.ha^{-1}$, and 10 $t.ha^{-1}$ (Afzalinejad *et al*., 2021). The *C. officinalis* was cultivated on approximately 100 m^2, with each plot consisting of three rows spaced 80 cm apart and measuring 4.5 m in length. Before sowing, composite soil samples were collected from the 0–30 cm depth of the field. After air-drying and passing the soil through a 2 mm sieve, important soil properties such as texture, pH, and electrical conductivity (EC) were determined (Table 1). The characteristics of the sewage sludge were also analyzed, focusing on heavy metals and nutrient content (Tables 2 and 3).

Table 1. Physical and chemical properties of the field soil

Factors studied	pH	EC ($ms\ cm^{-1}$)	Ca ($meq\ l^{-1}$)	K (ppm)	P (ppm)	N (%)	OC (%)	TNV (%)	SP (%)
Amount	7.56	5.18	29.8	520	288	0.31	1.7	28.7	27.67

Table 2. Heavy metals in Yazd municipal sewage sludge

Factors	pH	Mo ($Mg\ kg^{-1}$)	Se	As	Zn	Pb	Ni	Hg	Cu	Cr	Cd
Standard range	----	0.1-2.4	1.7-17.7	101-230	101-49000	13-26000	2-5300	----	84-17000	10-990	1-3.41
Amount	6.20	1.5	12	87	375	9	32	ND	147	16	1.28

Table 3. Nutrient composition of Yazd municipal sewage sludge

Factors studied	Mn	Fe	Bo	So	Na	Mg	Ca	K	P	N
	(Mg kg^{-1})			(%)						
Amount	125.8	5797	27.77	6	2.2	6.1	5.29	4.2	4.26	5.42

The *C. officinalis* seeds, Parpar Cultivar, were purchased from Pakan Bazr Company., Isfahan. Seeds were sown at a depth of 2 cm, with 15 cm spacing between plants in each row. After thinning, the plant density was adjusted to 20 plants m^{-2}. Growth performance factors were measured on 15 consecutive plants randomly selected from each plot while considering plot margins at the physiological maturity stage. These factors included plant height, number of flowers per plant, fresh weight, dry weight, flower yield, biological yield, flower diameter, capitula weight, capitula m^{-2}, seed count per capitula, 1000-seed weight, seed yield, seed harvest index, and the number of lateral branches per plant in each replication (Omidi *et al*., 2014). Seed yield was calculated as the ratio of seed weight to plant dry weight. To determine biological yield, three plants were sampled from each plot, and their total weight was measured. The average weight was then used to calculate the biological yield per plant. Quantitative color measurement of textiles is performed in the Lab color space. CIE $L^*a^*b^*$ (CIELAB) is the most complete color space introduced by the International Commission on Illumination. This space describes all colors visible to the human eye. The L^* component represents lightness or luminance, ranging from 0 to 100. While the parameters a^* (from green to red) and b^* (from blue to yellow) are two color components with a range of -120 to 120 (Yagoobi-Soureh *et al*., 2013). The assessment of lightfastness and washfastness, as well as the color spectroscopy of Pot marigold plants, was conducted at the Textile Chemistry Laboratory of Islamic Azad University, Yazd Branch. To determine the color values, the reflectance parameters a^*, b^*, and L^* were measured using a ColorEye7000A reflectance spectrophotometer with a D65 light source and a 10° viewing angle (manufactured by GretagMacbeth). A Heraeus LM Alpha Xenotest instrument, a 2-OC2-QE device, and an Atlas LHDEF washing fastness measurement device were used to measure light fastness and washing fastness, respectively.

First, waxes, fatty substances, and impurities were removed from the wool surface. The wool yarns used in this study were initially washed with a non-ionic soap. For the dyeing experiments, Iranian wool yarn with a metric count of 4.5 and 65 twists per meter was utilized (Shahparvari *et al*., 2019). The petals of the studied plants were separated and dried. Subsequently, dried Pot marigold petals (5 g) were weighed using a laboratory digital scale (with a precision of 0.001 g, model KERN, Germany) and mixed with 150 cc of distilled water in an Erlenmeyer flask. This mixture was placed on a laboratory heater for 10 minutes until the temperature reached 40°C, at which point 5 g of the wool yarn to be tested were added to the bath. The yarn was then boiled for 120 minutes at a temperature between 90 and 95°C (Shahparvari *et al*., 2019). The washfastness and lightfastness of the dyeing samples were assessed according to Iranian National Standards No. 10076 and 4084. To evaluate the washfastness of the dyed fibers, after dyeing and cooling, the colored fibers were washed with liquid soap (5 g) obtained from Henkel, Germany. Subsequently, it was rinsed with municipal water at 25°C before being dried. The samples were then analyzed for washfastness using a spectrophotometer in accordance with Iranian National Standard No. 10076 (ISIRI, 2007). To determine the lightfastness of the fibers, the dried fibers were twisted 20 times onto opaque cardboard (diameter of 1 mm and dimensions of 2 by 6 cm). They were exposed to direct sunlight for 8 hours each day for 20 days. The samples were then examined using the Macbeth Gretag 7000A spectrophotometer in accordance with Iranian National Standard No. 205 (ISIRI, 2015).

2.1. Data analysis

The normality of the data was first assessed. If the data were not normally distributed, normalization was performed. The data were analyzed using SPSS 20, and mean values were compared using Duncan's multiple range test at a significance level of 0.05.

3. Results and discussion

The analysis of variance for *C. officinalis* yield under different levels of wastewater sludge treatment (Table 4) exhibited that plant height and fresh weight per plant significantly differed among treatments ($p \leq 1\%$). Additionally, the number of flowers per plant and flower yield showed significant differences ($p \leq 5\%$). However, no significant differences were observed among treatments for dry weight, biological yield, and flower diameter.

Table 4. Analysis of variance for measured traits in *C. officinalis* under different levels of wastewater sludge treatment

S.O.V.	df	Mean square						
		Plant height	Number of flowers	Fresh weight	Dry weight	Flower yield	Biological yield	Flower diameter
Replication	2	1.7^{ns}	60.2^{ns}	774.2^{ns}	155.1^{ns}	4756.5^{ns}	735748.5^{ns}	0.02^{ns}
treatment	2	245.2^{**}	509.3^{*}	12343.2^{**}	561.9^{ns}	37879.1^{*}	2203134.7^{ns}	0.22^{ns}
Error	2	16.11	63.13	588.1	163.9	4271.5	625216.4	0.04
S.O.V.	df	Mean square						
		Single head weight	Number of heads per m^2	Number of seeds per head	1000 seed weight	Seed yield	Seed harvest index	Branch number
Replication	2	0.002^{ns}	61206.9^{**}	26.26^{ns}	11.8^{ns}	183309.4^{ns}	0.012^{ns}	22.4^{ns}
treatment	2	0.009^{ns}	36248.8^{**}	22.01^{ns}	14.1^{ns}	800869.5^{ns}	0.01^{ns}	23.7^{ns}
Error	2	0.003	1408.8	11.8	2.6	128122.2	0.005	12.8

(**) significant at the 1% level, (*) significant at the 5% level, and (ns) not significant at the 5% level among experimental treatments

The results indicated that dry weight per plant, flower diameter, single capitula weight, seed number per capitula, 1000-seed weight, grain harvest index, and lateral branches were not significantly affected by different levels of sewage sludge. However, Duncan's mean comparison revealed a significant increase in seed yield indices and 1000-seed weight, suggesting that the nutritional improvement and increased water availability from enhanced soil physical properties, resulting from sewage sludge application, may have promoted plant growth. Nevertheless, increased plant height might intensify intra-plant competition, potentially resulting in no notable changes in grain yield (Wenk and Falster, 2015). A study on the safflower plant also reported a non-significant increase in 1000-seed weight following fertilizer application. Field observations suggest that a high planting density in the experimental field caused no significant effect on the number of lateral branches (Chakerollhosseini, 2006). Koutroubas et al. (2021) found that, with increased plant density, competition for environmental resources intensified, leading to a significant reduction in lateral branches due to the limited availability of growth factors. Our results revealed that the potassium levels were found to be lower compared to other elements. Additionally, considering the soil analysis, it can be inferred that the insufficient potassium content in the added treatment may be one of the reasons for the non-significant grain harvest index. Excessive nitrogen loading in sewage sludge can stimulate unchecked vegetative growth, thereby compromising seed production. Conversely, potassium deficiency can adversely affect seed quality, impairing both seed formation and filling (Wenk and Falster, 2015). Other environmental factors, such as planting density and moisture, should also be considered in this context.

The mean comparisons of in number of flowers and fresh weight in *C. officinalis* under various levels of wastewater sludge at the $P \leq 0.05$ indicated a significant increase in these traits with the application of sludge at both 10 and 20 t.ha^{-1} (Fig. 1). Whereas the application of 10 t.ha^{-1} did not significantly affect plant height, while the 20 t.ha^{-1} treatment significantly increased this trait (Fig. 1).

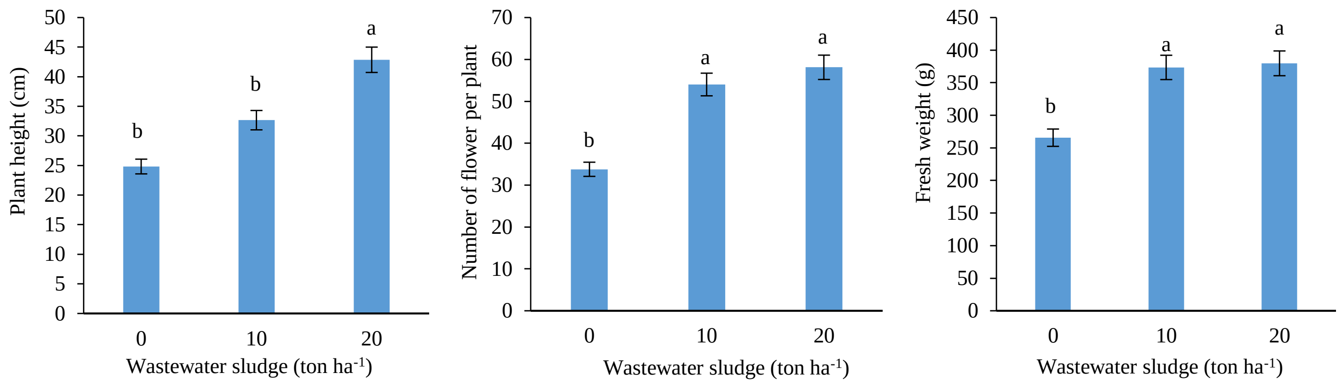

Figure 1. Mean comparison of plant height, number of flowers per plant, and fresh weight under various levels of wastewater sludge. Means with at least one shared letter are not significantly different according to Duncan's multiple range test at the 5% probability level.

Table 4 illustrates that the Number of heads per m^2 significantly differed at the 1% probability level. However, no significant differences were found for single capitula weight, seed count per capitula, 1000-seed weight, seed yield, seed harvest index, and number of lateral branches (Table 4). The mean comparison of flower yield per plant in *C. officinalis* under different levels of wastewater sludge indicated that the application of sludge, even at 10 t.ha^{-1}, increased both flower yield per capitula and flower diameter (p≤5%). However, this treatment exhibited no statistically significant difference compared to the control. It was revealed that the application of 20 t.ha^{-1} significantly enhanced both traits compared to the control. In terms of the capitulas number, the results showed that increasing the sludge level in treatments (control, 10, and 20 t.ha^{-1}) significantly affected this trait (Fig. 2). The mean comparison of 1000-seed weight and seed yield per plant in *C. officinalis* under different levels of wastewater sludge exhibited that sludge application, even at 10 t.ha^{-1}, increased the average 1000-seed weight. However, no statistically significant difference was observed for this treatment compared with the control. Notably, the application of 20 t.ha^{-1} significantly enhanced the mentioned parameter rather than the control (Fig. 3).

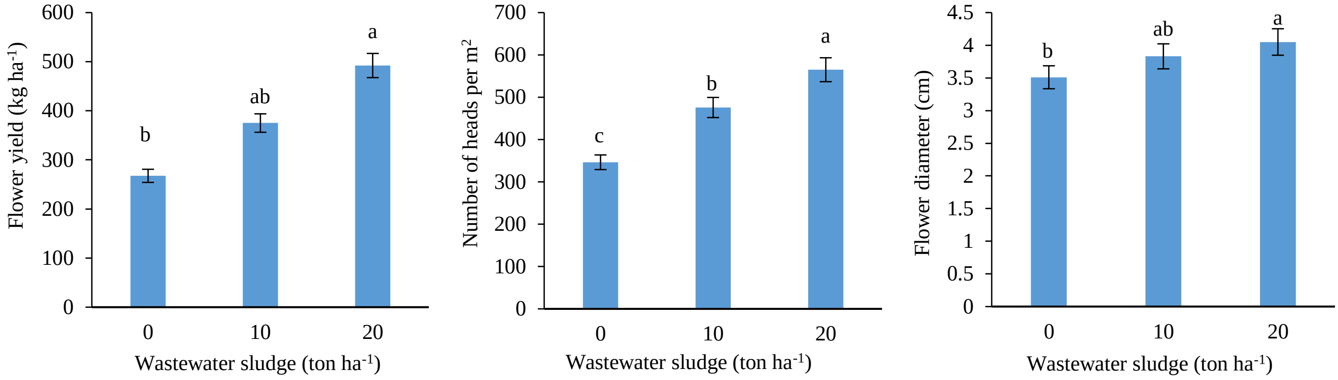

Figure 2. Mean comparison of flower yield, head number per m^2, and flower diameter of *C. officinalis* under different levels of wastewater sludge. Means with at least one shared letter are not significantly different according to Duncan's multiple range test at the 5% probability level.

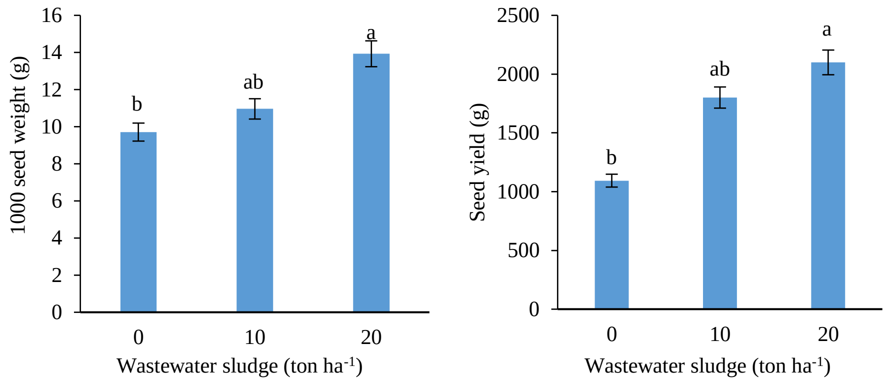

Figure 3. Mean comparison of 1000-seed weight and seed yield in *C. officinalis* under different levels of wastewater sludge. Means with at least one shared letter are not significantly different according to Duncan's multiple range test at the 5% probability level.

Results revealed that the application of 20 t.ha^{-1} of sludge significantly increased plant height, flower count per plant, fresh weight per plant, flower yield, biological yield, and Numbwe of Cpitula per m^2. Specifically, the 20 t.ha^{-1} treatment resulted in a 61% increase in plant height, 58% increase in flower count per plant, 42% increase in fresh weight per plant, 74% increase in flower yield, 47% increase in 1000-seed weight, and 84% increase in capitula count m-2. Due to the nutrient-rich composition of the used sludge, with total nitrogen exceeding 5%, it probably enhanced plant nutrition, leading to improved vegetative growth, increased photosynthesis, nutrient production, and yield (Fattahi *et al*., 2023). Furthermore, the addition of organic matter from wastewater sludge improves soil physical properties by increasing organic carbon content, reducing bulk density, and enhancing soil water-holding capacity and aggregate stability (Koutroubas *et al*., 2021). Similarly, Azimzadeh (2017) and Omidi et al. (2014) reported comparable results, suggesting that organic fertilizers promote vegetative growth and photosynthesis due to their nitrogen content and ultimately increase yield. Previous studies on safflower indicated that the application of 75 kg of nitrogen per hectare, a key element in wastewater sludge, optimized grain yield, plant height, and capitula count (Koutroubas *et al.*, 2021). Additionally, the increase in branch count and capitula number per plant contributes to greater plant height (Koutroubas *et al.*, 2021). Besides, a study on quinoa treated with wastewater sludge found that sludge significantly increased plant dry weight, attributed primarily to the considerable amounts of organic matter and essential nutrients present in the sludge (Afzalinejad *et al.*, 2021).

3.1. Absorption spectrum analysis of the solution containing extracted pigments from C. officinalis

The absorption spectrum of the color solution containing pigments extracted from *C.officinalis*, cultivated with 10 and 20 t.ha^{-1} of wastewater sludge, revealed a significant peak at 335 nm with an absorbance intensity of 0.75 (Fig. 4). The spectra indicate that sludge fertilization during the cultivation period significantly increased the absorbance peak intensity at this wavelength. The highest absorbance intensity was observed in the 10 t ha^{-1} fertilization regime compared to the 20 t ha^{-1} regime.

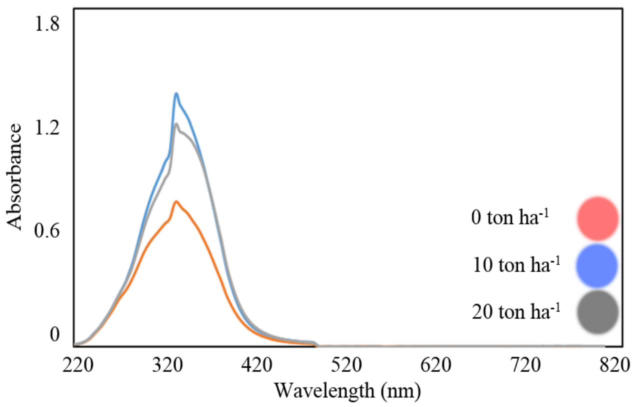

Figure 4. Absorption spectrum of the pigment solution extracted from *C. officinalis* under different sludge fertilizer treatments

The L* parameter, or lightness degree, of wool yarn dyed with the pigment extract from *C. officinalis* treated with different sludge levels (Table 5) revealed that using the extracted dyeing material reduced lightness (indicating a darker tone), reflecting increased dye uptake. The most considerable lightness reduction occurred in the control (no fertilizer), while the least reduction was seen in the 20 t.ha^{-1} sludge treatment. Furthermore, washing fastness measurements revealed that the 20 t.ha^{-1} treatment remained unchanged, demonstrating higher wash fastness than other treatments. However, for light fastness, the 10 t.ha^{-1} treatment exhibited the highest resistance to light stress, effectively preserving the dye in the fiber. For the a* parameter, indicating the green-red index, wool yarn dyed with the *C. officinalis* extract under different sludge treatments (Table 5) exhibited that sludge-treated dye at 20 and 10 t.ha^{-1} resulted in the highest red uptake compared to other levels. The 20-ton sludge treatment also showed the highest wash fastness among treatments. However, under light stress, the 10 t.ha^{-1} treatment exhibited greater resistance than other treatments, preserving the red hue of the dye in the fiber.

The b* parameter, indicating the blue-yellow index for wool yarn dyed with *C. officinalis* extract treated with varying levels of sludge (Table 5), showed that the dye material treated with 10 and 20 t.ha^{-1}of wastewater sludge yielded the highest yellowness values among the samples. Pot marigold plants fertilized with various levels of sewage sludge (0, 10, and 20 t.ha^{-1}) produced extracts that dyed raw wool with similar color attributes (L, a, b, ΔE, y, w) to the control treatment. The sludge may have provided essential nutrients, such as iron, zinc, or nitrogen, which enhanced dye uptake by the plants, especially at the 10 t.ha^{-1} application rate (Lyu *et al.*, 2022). However, at 20 t.ha^{-1} treatment, selective nutrient absorption was observed, resulting in a reduced dye uptake peak (Guesmi *et al.*, 2012).

Under washing stress, all treatments demonstrated similar resistance levels, showing minimal reduction. However, under light stress, the 20 t.ha^{-1} sludge treatment exhibited superior performance in maintaining the dye's yellow hue within the fiber. The Y parameter, representing the yellowness index of wool yarn dyed with *C. officinalis* extract treated with different sludge levels (Table 5), indicated that using sludge-treated dye at both 20 and 10 t.ha^{-1} yielded the highest yellowness values compared to other samples, closely aligning with the b* index results. The W parameter, which represents the whiteness index of wool yarn dyed with *C. officinalis* extract under different sludge treatments (Table 5), also showed that the dyeing sample treated with 20 t.ha^{-1} sludge had the lowest whiteness of all samples. This indicates more effective dye uptake compared to other treatments. Besides, the ΔE color difference index, calculated to assess the color variation between the dyed samples and the undyed control yarn with *C. officinalis* extract (Table 5), demonstrated that the control sample exhibited the highest color difference from the untreated wool yarn. In contrast, other samples displayed values close to the control. Furthermore, ΔE calculations assessing color variation under different fertilization treatments with *C. officinalis* showed that the 10 $t.ha^{-1}$ treatment produced the least variation in color after washing stress. This result indicates a higher washing fastness than other samples. Regarding light fastness calculations using the ΔE index, the 20 $t.ha^{-1}$ sludge treatment displayed the lowest color variation under light stress, demonstrating the highest resistance among all treatments.

Table 5. Color properties of wool yarn dyed with pigments extracted from *C. officinalis* cultivated under different sludge treatments

treatment		L^*	a^*	b^*	Y	W	ΔE compared to raw sample	ΔE compared to dyed sample
Raw wool yarn		94.4	3.23	21.5	44.5	-11.16	-	-
Dyed yarn with Pot marigold Control	Dyeed	94.9	0.96	26.1	48.8	-3	5.1	---
	Washing stress	94.7	0.88	27.6	50.8	-37	1.8	1.5
	Light stress	87.6	16.3	16.9	50.7	-7	18	19.3
Dyed yarn with Pot marigold Treatment 10 T h^{-1} dyed sludge	Dyeed	94.5	1.15	26.9	50.4	-34	5.6	---
	Washing stress	94.9	0.73	27.5	50.5	-36	1	1.37
	Light stress	87.5	16.5	17.2	51.4	-9	19	19.3
Dyed yarn with Pot marigold Treatment 20 T h^{-1} dyed sludge	Dyeed	93.7	2.9	26.6	51.5	-36	5.2	---
	Washing stress	93.7	2.6	27.6	52.5	-40	1	2.5
	Light stress	88.1	15.7	17.4	50.8	-8.6	17	18.3

In calendula plants grown in soil with 10 and 20 $t.ha^{-1}$ of sewage sludge, notable improvements were observed in stress tolerance traits, such as photobleaching resistance and washability and This improvement is likely attributed to the enrichment of the plant's biochemical composition under the influence of sewage sludge. Sewage sludge is a rich source of micronutrients such as iron, manganese, zinc, and copper, all of which play essential roles in the biosynthesis of plant pigments, particularly carotenoids and flavonoids (Nikghadami *et al*., 2020). Enhanced availability of these elements may lead to increased production and stability of such pigments within the plant tissues.

Moreover, the organic matter present in sewage sludge contributes to better nutritional conditions for the plant, which in turn promotes the synthesis of phenolic and antioxidant compounds. These metabolites not only contribute to color intensity but also improve the stability of the dye against degrading factors such as light and washing agents (Shamsnateri and Jafari, 2007). Additionally, the improved growing conditions may stimulate the formation of more complex pigment structures or the co-production of co-pigments, both of which can result in enhanced dye fastness (Table 5).

This result can be due to the uptake of metals and elements from the sludge, which enhances dye properties by forming complexes with metal ions present in the plant (Nikghadami *et al*., 2020) Mordants, which contain metal ions, are essential for enhancing the fastness properties (e.g., wash and abrasion resistance) of natural dyes on wool. Metal ions facilitate the formation of complexes between dye molecules and the protein chains in wool, improving dye attachment and overall fastness (Shamsnateri and Jafari, 2007). Furthermore, the binding of these metal ions with the aforementioned dye compounds alters their color. Additionally, the interaction between the metal ions and the fibers enhances the fastness properties of the dyed fabric.

4. Conclusion

The plant's yield response was evaluated across six factors: fresh weight per plant, flower yield, biological yield, flower number per plant, capitula per m^{-2}, and plant height. An increase was observed with the application of sewage sludge, with the highest performance reported at the treatment of 20 $t.ha^{-1}$ of sewage sludge. Key contributors to the elevated performance metrics include the effective presence of nitrogen and phosphorus, as well as moisture retention in the soil facilitated by the organic matter in the sludge. Additionally, the application of sewage sludge significantly affected the dye quality obtained from the plants, with the highest absorption peak recorded in the extracts from plants treated with 10 $t.ha^{-1}$ of sewage sludge. Dyeing with extracts from sludge-treated plants resulted in a notable increase in light and wash fastness due to the presence of metal ions in the plants, which strengthened their bond with the dye molecules. Therefore, cultivating calendula with 20 $t.ha^{-1}$ of sewage sludge is recommended for dyeing purposes. However, further research is needed to investigate its use in medicinal and nutritional applications, particularly concerning the accumulation of heavy metals in the plant and its products.

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No humans or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

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