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Impact assessment of heavy metals spi ked wastewater irrigation on growth, keeping quality and productivity of Calendula (*Calendula officinalis* L.)

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Abstract

A pot experiment was conducted in the net house of the research farm area of the Water Technology Centre of ICAR-IARI New Delhi during the year 2021-22. Seven treatments T-1: Sole Groundwater Irrigation without spiking of heavy metals, T-2: Sole Wastewater Irrigation without spiking of heavy metals, T-3: Wastewater Irrigation spiked with Cd (0.005ppm), Cr (0.05ppm), Ni (0.1ppm) and Pb (2.5ppm),T- 4: Wastewater Irrigation spiked with Cd (0.01ppm), Cr (0.1ppm), Ni (0.2ppm) and Pb (5.0ppm), T-5:Wastewater Irrigation spiked with Cd (0.1ppm), Cr (1ppm), Ni (2ppm) and Pb (10ppm), T-6:Wastewater Irrigation spiked with Cd (0.25ppm), Cr (2.5ppm), Ni (5ppm) and Pb (30ppm),T-7: Wastewater Irrigation spiked with Cd (0.5ppm), Cr (5.0ppm), Ni (10ppm) and Pb (50ppm) were taken under 3 replications in a Completely Randomized Design (CRD) with three replications. The local selection variety of Calendula (*Calendula officinalis* L.) was used as the test crop. Standard methods were followed to record the necessary observations. Results indicated that vegetative growth parameters of calendula were significantly altered while keeping quality of calendula were not found deterioted under metals spiked wastewater. Calendula flower yield obtained was higher in the treatments under heavy metals spiked wastewater. However, long-term application of heavy metals rich wastewater in calendula needs to be monitored at different soil and climatic conditions.

Key words : Calendula, growth, heavy metals, irrigation, productivity, wastewater.

Introduction

India, accounting for approximately 2.45 percent of the world's surface area and accommodating 17 percent of the global population, faces a significant challenge with its water resources, constituting only 4 percent of the world's total. The rapid rise in population has resulted in increased urbanization and industrialization, leading to a scarcity of freshwater for agricultural purposes. The predominant usage of freshwater for domestic, commercial, and industrial needs has amplified wastewater generation (Qadir *et al.*, 2010). Regrettably, only 24% of the wastewater produced by households and businesses in India undergoes proper treatment before being utilized in agriculture or released into rivers (Minhas and Samra, 2003). Many developing cities lack adequate wastewater treatment facilities, further exacerbat-

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ing the situation (Qadir *et al.,* 2010). Consequently, urban and peri-urban farmers resort to using partially treated, diluted, or untreated wastewater to cultivate diverse crops (Ensink *et al.,* 2007). The deliberate adoption of wastewater irrigation by farmers in urban and industrial regions, primarily for high-value crops like vegetables and cereals, stems from its numerous benefits. These advantages include higher nutrient content such as nitrogen, phosphorus, potassium, and essential micronutrients, along with organic carbon, greater availability, lower energy requirements, and cost-effectiveness compared to other irrigation methods (Kaur *et al.,* 2012; Lal *et al.,* 2013). However, the practice of wastewater irrigation also poses potential hazards to soil health and the plants irrigated due to the accumulation of excessive nutrients and metals in the soil. Wastewater contains varying amounts of heavy metals like copper (Cu), cadmium (Cd), zinc (Zn), lead (Pb), nickel (Ni), and chromium (Cr), which may contaminate the food chain when used for irrigation. Long-term application of wastewater irrigation can lead to the build-up of heavy metals in the soil, which persistently affects their concentration, availability, and uptake by plants (Rattan *et al.,* 2005; Singh *et al.,* 2004). Consequently, it is essential to raise awareness about the issue of metal contamination in the food chain caused by wastewater irrigation to safeguard public health and the environment (Ciura *et al.,* 2005). A potential solution for the sustainable and safe utilization of wastewater lies in its use for non-edible crops, such as flowers. Floral cultivation minimizes the risk of heavy metal contamination in the food chain, while flowers themselves can serve as effective phytoremediators, removing heavy metals from soils contaminated by wastewater irrigation (Ciura *et al.,* 2005). Compared to costly chemical or physical remediation methods, phytoremediation offers a low-tech, cost-effective, and environmentally friendly approach to address heavy metal pollution. In phytoremediation, plants act as bioreactors, facilitating the transfer, bioaccumulation, and degradation of contaminants through their shoots and pollutant-uptake capacities in their roots (Mani *et al.,* 2016). The selection of suitable plants for phytoremediation relies on their resistance to toxicity and affinity for metals, while ongoing research focuses on soil quality improvement and plant selectivity. Traditional remediation techniques involving soil extraction and physical, thermal, or chemical treatments are often expensive, highlighting the promise of phytoremediation as an economical and effective method for soil decontamination and pollutant removal (Mani *et al.,* 2016). Phytoremediation demonstrates its versatility in various settings, from small-scale brownfields to extensive mining landscapes, and has proven successful in cleaning excavated river sediments (Mani *et al.,* 2016). Considering these factors, calendula (*Calendula officinalis L. var. local selection*) was selected as the test crop for assessing its cultivation feasibility under irrigation with heavy metals rich wastewater in the present study.

Materials and Methods

The pot experimental study was carried out under calendula (*Calendula officinalis L. var. local selection*) in the net house of research farm area of the Water Technology Centre (28° 37' 22" to 28 °39' 00" N latitude and 77° 8' 45" to 77° 10' 24" E longitudes with an average elevation of 230m above mean sea level) of ICAR- Indian Agricultural Research Institute (IARI), New Delhi, India during the year 2021-22. The study area is a part of the 6th Agro-Climatic Region Zone (Trans-Gangetic Plains Region) and 4th Agro-Ecological Region (Host semi-arid eco-region with alluvium derived soil) of India has a subtropical and semi-arid climate with hot, dry summer and cold winter. The long-term (past 30 years) average annual rainfall was 710 mm. The initial soil taken to fill the pots was sandy loam. The pH_2 (6.48) and EC_2 (0.22 dS/m) were optimum. Mean soil organic carbons (0.21%), available N (143.2 kg/ha), available P (40.7 kg/ha) were low, and available K (586.4 kg/ha) was high. Moisture retention at field capacity (-33 kPa) was (-33 kpa) was 22.5% (w/w) and at permanent wilting point (-1500 kPa) was 8.0% (w/w). The bottom holed light colored plastic pots (size: Upper Diameter- 25 cm, Lower diameter: 12.5 cm and Height: 30 cm) with a capacity of 5 liters were used to plant the calendula (*Calendula officinalis* L. *var. local selection*) seedlings. The 5 kg soil well crushed and sieved was used to fill the pots to ensure an optimum bulk density of 1.50g/cc. Recommended dose of urea, single super phosphate and muriate of potash were mixed thoroughly in the soil before filling the pots. Seven treatments T-1: Sole Groundwater Irrigation without spiking of heavy metals,T-2: Sole Wastewater Irrigation without spiking of heavy metals,T-3: Wastewater Irrigation spiked with Cd (0.005 ppm), Cr (0.05ppm), Ni (0.1ppm) and Pb (2.5ppm), T- 4: Wastewater Irrigation spiked with Cd (0.01ppm), Cr (0.1ppm), Ni (0.2ppm) and Pb (5.0ppm), T-5: Wastewater Irrigation spiked with Cd (0.1ppm), Cr (1ppm), Ni (2ppm) and Pb (10ppm),T-6: Wastewater Irrigation spiked with Cd (0.25ppm), Cr (2.5ppm), Ni (5ppm) and Pb (30ppm),T-7: Wastewater Irrigation spiked with Cd (0.5ppm), Cr (5.0ppm), Ni (10ppm) and Pb (50ppm) were taken under 3 replications in a Completely Randomized Design. The irrigation was scheduled on the basis of 50% depletion of available soil moisture depletion (DASM) with an irrigation depth of 5cm. Nitrate salts of cadmium (Cd $(NO_3)_2.4H_2O$), chromium (Cr $(NO_3)_3.9H_2O$), nickel (Ni $(NO_3)_2.6H_2O$) and lead (Pb $(NO_3)2$) were used for preparation of desired level of heavy metals spiked wastewater. Calculation of salts for preparation of desired level heavy metals spiked wastewater is given in Table 1. Municipal wastewater was collected from the drainage channel passing near WTC farm while groundwater was collected from Tube well after the submersible pump was in operation for about half an hour, located near field no. WTC-1 in the eight plastic tanks (Capacity Size: 50 liters) represented the seven treatments from T-1 to T-7. Water samples of both the waters were analyzed for different water quality parameters as per standard methods. All the water samples have pH values ranging from 7.02 to 7.84, indicating slightly alkaline water. Salinity in terms of EC of water samples ranged from 0.45 to 2.06 dS/m, with the groundwater having the lowest salinity. All the water was within the pH and EC safe limits, and SAR values were determined using the concentrations of Na^+, Ca^{2+}, and Mg^{2+} and these were below the permissible limit (SAR < 10). There were no RSC issues in any of the treatment water. Total NPK concentrations in irrigation water ranged

Table 1. Calculation of heavy metal salts for preparation of desired concentration of metals for heavy metals spiked wastewater (2020-21).

Heavy Metals	Treatment Conc. (ppm)	Amount of salt required for 1 L water to make 1 ppm metal solution (mg)	Amount of salt required for 1 L water to make desired ppm concentration solution of metal (mg)	Amount of salt required for 50 L water to make desired ppm concentration solution of metal (mg)
Cd	0.005	2.74	0.014	0.7
	0.01	2.74	0.027	1.4
	0.1	2.74	0.274	13.7
	0.25	2.74	0.686	34.3
	0.5	2.74	1.372	68.6
Cr	0.05	7.70	0.385	19.2
	0.1	7.70	0.770	38.5
	1	7.70	7.696	384.8
	2.5	7.70	19.239	962.0
	5	7.70	38.479	1923.9
Ni	0.1	4.95	0.495	24.8
	0.2	4.95	0.991	49.5
	2	4.95	9.909	495.4
	5	4.95	24.772	1238.6
	10	4.95	49.544	2477.2
Pb	2.5	1.60	3.996	199.8
	5	1.60	7.992	399.6
	10	1.60	15.985	799.2
	30	1.60	47.955	2397.7
	50	1.60	79.925	3996.2

from 10.2 to 34.45 mg/L, 0.22 to 1.94 mg/L, and 1.20 to 2.56 mg/L. Total lead (Pb), cadmium (Cd), nickel (Ni), and chromium (Cr) concentrations in all water samples ranged from ND to 50.0, 0.00 to 0.50, 0.01 to 10.00, and 0.00 to 5.00 mg/L. Biometrical observations on experimental calendula were recorderd as mean leaf length (Leaf length of mid part of plant is being measured at vegetative and reproductive stages of calendula using Vernier calipers), mean leaf width (Leaf width of calendula is measured at the mid plant part during vegetative and reproductive stages of calendula), no. of leaves/plant (just counting the leaves manually), plant height (Plant height is being measured from soil surface to apex flower bud), flower bud initiation (Days to initiation of first flower bud is measured from date of planting to date of first flower bud initiation), no. of flowers/plant (Flowers per plant is counted through counting of flowers picked/harvested at each picking or harvesting), mean diameter of open flower (Diameter of open flower will be measured at the time full bloom stage of calendula/picking stage), fresh flower weight (It is measured as average weight of 3 full bloom flowers in each treatment pot), flower dry weight. (It is measured as average weight of 3 full bloom flowers in each treatment pot), fresh plant weight (shoot+ root) (Total weight of plant after harvesting), dry plant weight (shoot +root) (Total weight of oven dried plant) flower durability in field (Field flower durability is counted from the date of full bloom flower to the date of drying the same flower at field condition), vase life of single flower (Vase life of single flower is measured from date of picking/harvesting of full bloom calendula flower and keep at room temperature and note the date of drying of flower). All data on growth, keeping quality and productivity of calendula (*Calendula officinalis L. var. local selection*) were statistically analyzed by the analysis of variation (ANOVA) technique. ANOVA was carried out on the data for each parameter as applicable to a completely randomized design (Gomez and Gomez, 1983). The significance of the treatment effect was determined using F-test, and to determine the significance of the difference between the means of the two treatments, the least significant differences (LSD) were estimated at a 5% probability level. Duncan's multiple range test was used for comparing three or more means at the same probability level.

Results and Discussion

The results and discussion on impacts of heavy metals spiked wastewater irrigation on growth, keeping quality and productivity of calendula (*Calendula officinalis L. var. local selection*) are described below with following sub-heads.

Impact on vegetative growth parameters of Calendula

It is revealed from Table 2 that the pots irrigated with wastewater (95.33) had higher number of leaves in comparison to the groundwater irrigated plants (75.33). The number of leaves has differed significantly over the treatments under heavy metals spiked wastewater (T-3 to T-7). Highest shoot length was observed in T-7, while

Table 2. Impact of metal spiked wastewater irrigation on vegetative growth parameters of calendula (*Calendula officinalis* L.)

Treatment	Shoot length (cm)	Root length (cm)	Leaves/ plant	Mean leaf length (cm)	Mean leaf width (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)
T1	33.27	40.63	76.33	11.85	4.29	28.67	8.39	36.33	7.70
T2	34.70	38.70	95.33	15.62	3.47	42.00	11.87	54.33	11.87
T3	42.30	42.83	110.00	13.17	3.88	50.67	17.66	44.67	10.04
T4	42.87	32.57	115.33	14.84	3.44	50.33	13.55	46.67	11.54
T5	38.97	40.60	119.33	13.90	4.09	53.67	14.85	52.33	13.19
T6	41.60	53.70	127.33	14.15	3.78	78.33	31.27	58.33	14.50
T7	44.83	46.63	118.33	14.53	3.79	85.00	23.18	65.33	16.44
SE(m)	2.725	4.564	5.674	1.045	0.383	4.465	2.213	5.270	1.298
CD (P=0.05)	NS	NS	17.376	NS	NS	13.674	6.777	16.141	3.975

the highest root length was found in T-6. The calendula mean leaf length under wastewater irrigation was higher than the groundwater irrigated plant. This could be due to the higher concentration of NPK content in wastewater as compared to the groundwater. Alghobar (2006) observed higher leaf length in napier grass irrigated with treated and untreated wastewater as compared to the groundwater irrigation. There was non-significant difference in the mean leaf width of treatments under heavy metals spiked wastewater, the treatment under groundwater irrigation had only slight increase in the mean leaf due to the environmental factors and the time of first flower picking when this observation was recorded. The fresh plant weight (shoot + root) of calendula obtained from wastewater irrigation was much higher (48.2 percent) than that of groundwater irrigated plants. This is mainly because of the higher NPK content in wastewater. Looking at the results it may be noted that the plant fresh weight did not decrease with the increase in the heavy metal concentration. This is evident from the fact the relative density of heavy metals is very high > 5 g/cm^3 (Raychaudhuri *et al.,* 2021) which tend to accumulate in the plant parts thus contributing to the increased biomass. Similarly, the dry plant weight was found to be following the same trend as for the fresh plant weight.

Impact of heavy metals spiked wastewater on flowering, & keeping quality parameters and yield of Calendula

It is evident from the Table 3 that there was no significant variation in the number of days taken for the first flower bud initiation in the calendula pot experiment. However, the flower yield obtained from the wastewater irrigation was higher than the groundwater irrigated pots. The yield increased with the increase in heavy metals spiked wastewater. This could be due to increase in Nickel concentrations over the treatments. Zahara and Sobati (2021), studied the effect of foliar application of Nickel on calendula and reported that it had a positive effect on phytochemical characteristics of calendula pot flowers. T-7 had recorded the highest flower yield (37) as against the groundwater treated pot T-1 (14.33) and sole wastewater treated T-2 (16). The graphs related to these flowering parameters are presented from Figure. 4.10 to 4.15 respectively. It is also revealed from Table 3 that there was no significant difference in the keeping quality of T-1 and wastewater irrigated T-2 to T-7. The flower durability in field of groundwater irrigated pot (T-1), wastewater irrigated pot (T-2) and treatments with heavy metals spiked wastewater (T-3 to T-7). Similar trend was observed for the vase life duration for the treatments. This could be useful in the sense that the metals spiked wastewater did not deteriorate the keeping quality of calendula. It is also clear from the Table 3 that the effect of heavy metals spiked wastewater on the flower diameter and weight of single flower was non-significant. Thus the heavy metal application did not have any negative impact on the flower diameter.

Table 3. Impact of metal spiked wastewater irrigation on flowering and keeping quality parameters of calendula (*Calendula officinalis* L.) *cv.* Local Selection (2021-22).

Treatment	Mean flower bud initiation (days)	flower/ plant (No)	Diameter of open flower (cm)	Weight of single flower (g)	Flower durability (days)	Vase life (days)
T-1	78.667	14	5.37	1.96	6.44	7.00
T-2	84.333	16	5.60	2.72	6.44	6.78
T-3	79.667	20	5.22	2.04	6.89	6.78
T-4	78.667	24	5.36	2.02	7.22	6.56
T-5	75.000	26	6.14	2.79	7.44	7.67
T-6	75.333	33	6.07	2.65	7.11	6.78
T-7	76.667	37	5.48	1.96	7.67	6.89
SE(m)	2.059	3.122	0.314	0.434	0.915	0.841
CD (P=0.05)	NS	9.561	NS	NS	NS	NS

Conclusion

It is concluded from the study that vegetative growth parameters of calendula were significantly altered while keeping quality of calendula were not found deterioted under metals spiked wastewater. Calendula flower yield obtained was higher in the treatments under heavy metals spiked wastewater. However, long-term application of heavy metals rich wastewater in calendula needs to be monitored at different soil and climatic conditions.

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