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EFFECT OF GRADED LEVELS OF NICKEL AND ZINC ON SOIL PROPERTIES, METAL BIOAVAILABILITY, BIOMASS PRODUCTION AND HEAVY METAL UPTAKE IN MARIGOLD (*TAGETES ERECTA* L.)

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ABSTRACT

Heavy metal enrichment in agricultural soils is increasing due to anthropogenic activities, often affecting plant growth and soil processes. Among essential heavy metals, nickel (Ni) and zinc (Zn) function as micronutrients but become toxic at elevated concentrations. This study evaluated the effect of graded Ni and Zn levels on soil properties, metal availability, biomass accumulation, and uptake in marigold (*Tagetes erecta* L.) under pot culture. Eight treatments comprising control, three Ni levels (0.2–2 mg L^{-1}), three Zn levels (2–10 mg L^{-1}), and a combined Ni+Zn treatment were arranged in a Completely Randomized Design. Post-harvest soil analysis indicated that pH remained stable but EC and organic carbon varied significantly across treatments. DTPA-extractable Ni and Zn increased proportionally with their applied doses, with the highest availability recorded in the combined treatment. Metal concentrations and uptake in shoots and flowers showed strong dose-dependent increases, with synergistic enhancement under dual metal exposure. Moderate Zn improved biomass production, whereas higher levels of both metals reduced growth. The study demonstrates marigold's strong ability to accumulate Ni and Zn, confirming its potential as a bioindicator and phytoremediation species for metal-impacted environments.
***Keywords*:** Marigold, Nickel, Zinc, Biomass yield, Heavy metal uptake, Phytoremediation

Introduction

Heavy metal accumulation in agricultural ecosystems has increased rapidly due to anthropogenic activities such as industrial effluents, solid waste disposal, agrochemical misuse, vehicular emissions, and the long-term application of sewage and wastewater for irrigation. Among heavy metals, nickel (Ni) and zinc (Zn) are notable because they function both as essential micronutrients and potential toxicants depending on their concentration in the soil–plant system (Kabata-Pendias, 2011). Nickel is involved in several metabolic pathways and is an integral component of the urease enzyme, while zinc plays a crucial role in enzyme activation, protein synthesis, membrane integrity, and auxin regulation (Broadley *et al*., 2007). However, excess Ni induces chlorosis, oxidative stress, and membrane deterioration, whereas elevated Zn causes metabolic inhibition, nutrient imbalance, and reduced biomass (Cakmak, 2000; Yadav, 2010; Yusuf *et al*., 2011).

Understanding the behavior of Ni and Zn in soils, as well as their uptake and translocation within plants, is essential for sustainable nutrient management and environmental protection. Ornamental crops, especially those irrigated with wastewater, are particularly vulnerable to heavy metal accumulation yet receive limited scientific attention compared to food crops. Marigold (*Tagetes erecta* L.) is an economically significant ornamental species valued for landscaping, loose flower production, and carotenoid extraction. Its fast growth, high biomass, and tolerance to moderate heavy metal stress make it a suitable model for studying soil–metal–plant interactions and phytoremediation potential (Bosiacki and Wojciechowska, 2012).

The present study investigates the impact of graded concentrations of Ni and Zn on soil properties, metal bioavailability, tissue metal accumulation, biomass production, and metal uptake in marigold grown under non-polluted soil conditions. The findings are expected to contribute to research in environmental chemistry, soil fertility management, ornamental horticulture and phytoremediation under increasing heavy metal exposure.

Materials and Methods

Pot experiment and treatment details

The present study was conducted during 2023-24 at the research farm of Centre for Protected Cultivation Technology, ICAR-Indian Agricultural Research Institute, New Delhi. Marigold (*Tagetes erecta* L.) cv. Pusa Bahar was selected due to its adaptability, uniform flowering, and suitability for pot studies. Surface soil (0–15 cm) was collected from ICAR–IARI farm, New Delhi (28°38'6.3" N, 77°08'56.3" E). The soil was air-dried, crushed, sieved (2 mm), and homogenized.. Four kilograms of processed soil were filled into 8-inch UV-stabilized plastic pots. A basal fertilizer dose equivalent to 100:80:80 kg ha^{-1} N:P:K was applied using urea, DAP, and MOP. One-month-old seedlings were transplanted. Irrigation water containing respective Ni and/or Zn concentrations was supplied regularly. A Completely Randomized Design (CRD) was used with eight treatments **and** three replications. The treatment details are: T1: Control, T2: Ni @ 0.2 mg L^{-1}, T3: Ni @ 1.0 mg L^{-1}, T4: Ni @ 2.0 mg L^{-1}, T5: Zn @ 2 mg L^{-1}, T6: Zn @ 5 mg L^{-1}, T7: Zn @ 10 mg L^{-1}, T8: Ni @ 2 mg L^{-1} + Zn @ 10 mg L^{-1}. Metal salts used were- $Ni(NO_3)_2·6H_2O$ and $ZnSO_4·7H_2O$. The recorded data were analyzed statistically using ANOVA through the Web-Based Agricultural Statistics Software Package 2.0 (WASP 2.0).

Determination of soil and plant properties

Soil pH was measured in a 1:2 soil–water suspension using a digital pH meter, and EC was determined in the supernatant of the same extract using a conductivity meter (Jackson, 1973). Soil organic carbon was estimated by the Walkley and Black wet oxidation method (Walkley & Black, 1934). Available nitrogen was determined by the alkaline $KMnO_4$ method (Subbiah & Asija, 1956). Available phosphorus was analyzed using the Bray and Kurtz ascorbic acid method (Bray & Kurtz, 1945), while available potassium was extracted with 1N ammonium acetate and measured using a flame photometer (Hanway & Heidel, 1952). Available Zn and Ni of initial and post-harvest soil was determined using DTPA–$CaCl_2$–TEA extraction method following Lindsay and Norvell (1978) and quantified by ICP-MS (Perkin Elmer NexION 300). For determination of total metal content in initial and post-harvest soil aqua-regia digestion of soil samples was performed as per Quevauviller (1998), and metals (Zn and Ni) were analyzed by ICP-MS. Dried plant and flower samples were microwave-digested with HNO_3, and Zn and Ni concentrations were estimated using ICP-MS. Metal uptake was calculated by multiplying shoot or flower biomass with corresponding metal concentration.

Results and Discussion

Soil physico-chemical properties before experimentation

The initial soil of the IARI site exhibited a slightly alkaline pH (8.14) and a low EC (0.44 dS m^{-1}), indicating non-saline conditions suitable for nutrient availability and normal root functioning. The organic carbon content (0.62%) reflected moderate soil fertility. Available macronutrients- N (227 kg ha^{-1}), P (25 kg ha^{-1}), and K (371 kg ha^{-1})- were within agronomically acceptable ranges. Although available Zn (1.20 mg kg^{-1}) and Ni (0.35 mg kg^{-1}) appeared low, the total metal concentrations of Zn (150 mg kg^{-1}) and Ni (18.1 mg kg^{-1}) were relatively high, confirming the presence of a significant non-bioavailable heavy metal pool. Such a scenario is chemically relevant because metals strongly bound to soil colloids or carbonates may become mobilized under changes in pH or redox conditions.

Post-harvest soil properties

Post-harvest soil analysis revealed that pH remained consistent and non-significant (7.80–7.83) across treatments (Table 1), indicating that the applied Ni and Zn concentrations were insufficient to modify the inherent buffering capacity of the soil. Such stability is characteristic of alkaline soils, which tend to resist pH fluctuations even under metal-induced stress due to their high carbonate content and strong base saturation (Alloway, 2013; McBride, 2003; Kabata-Pendias, 2011). In contrast, EC values exhibited significant variation, ranging from 0.12 dS m^{-1} in T5 (Zn @ 2 mg L^{-1}) to 0.17 dS m^{-1} in T8 (Ni @ 2 mg L^{-1} + Zn @ 10 mg L^{-1}). The increased EC in metal-enriched treatments reflects the accumulation of soluble ions released from the applied metal salts, corroborating earlier findings that external metal inputs enhance ionic strength in the soil matrix (Lindsay and Norvell, 1978; Wuana and Okieimen, 2011).

Organic carbon content also responded distinctly to treatments. Low to moderate metal levels, particularly Ni @ 0.2 mg L^{-1}, Zn @ 2 mg L^{-1} and Zn @ 5 mg L^{-1}, and (T2,T5 and T6 , resulted in higher OC values (0.78% and 0.77%), likely attributable to improved root proliferation and rhizo-deposition under lower stress conditions (Marschner, 2012). In contrast, the combined high-dose treatment (T8: Ni @ 2 mg L^{-1} + Zn @ 10 mg L^{-1}) reduced OC (0.65%), suggesting microbial inhibition or reduced root turnover under intensified metal toxicity. Similar reductions in soil organic carbon under heavy metal–rich irrigation sources were reported by Verma *et al.* (2022), who demonstrated that metal-laden wastewater adversely affected soil biological processes and carbon cycling.

DTPA extractable Ni and Zn content varied significantly across treatments. Control soil (T1) exhibited the lowest extractable Ni (0.31 mg kg^{-1}) and Zn (1.16 mg kg^{-1}). Increasing Ni application elevated extractable Ni to 13.80 mg kg^{-1} in Ni @ 2 mg L^{-1} (T4). Zn treatments markedly increased extractable Zn from 11.20 mg kg^{-1} (T5: Zn @ 2 mg L^{-1}) to 46.10 mg kg^{-1} (T7: Zn @ 10 mg L^{-1}). The highest extractable Ni (14.20 mg kg^{-1}) and Zn (48.80 mg kg^{-1}) were observed in the combined treatment (T8: Ni @ 2 mg L^{-1} + Zn @ 10 mg L^{-1}), demonstrating a synergistic enhancement of metal availability. Verma *et al.* (2022) also reported that wastewater containing elevated heavy metal salts tends to accumulate in soils over time, increasing extractable metal pools and mobility. This is chemically plausible because co-application of metals can modify sorption dynamics, disrupt surface complexation equilibria, and increase the proportion of DTPA-chelatable metal fractions.

Table 1: Effect of application of graded concentration of Ni and Zn on post-harvest soil properties and DTPA extractable metals.

TREATMENT		**Post-harvest soil properties**			**DTPA extractable metals (mg kg^{-1})**	
		pH	**EC (ds/m)**	**Organic Carbon (%)**	**Ni**	**Zn**
Control	T1	7.80	0.13^{e}	0.61^{e}	0.31^{c}	1.16^{d}
Ni@0.2 mg/L	T2	7.81	0.13^{e}	0.77^{a}	2.04^{c}	1.24^{d}
Ni@1 mg/L	T3	7.83	0.14^{d}	0.73^{b}	7.47^{b}	1.32^{d}
Ni@2mg/L	T4	7.83	0.15^{c}	0.68^{c}	13.80^{a}	1.44^{d}
Zn@2mg/L	T5	7.80	0.12^{f}	0.78^{a}	0.33^{c}	11.20^{c}
Zn@5mg/L	T6	7.81	0.14^{d}	0.76^{a}	0.36^{c}	24.60^{b}
Zn@10mg/L	T7	7.82	0.16^{b}	0.70^{bc}	0.40^{c}	46.10^{a}
Ni@2 mg/L +Zn@10mg/L	T8	7.83	0.17^{a}	0.65^{d}	14.20^{a}	48.80^{a}
CD (0.05)		**N/A**	**0.01**	**0.03**	**1.74**	**2.70**

Heavy metal content in shoot and flower of marigold

Nickel and zinc accumulation in marigold tissues displayed a distinct dose-dependent response to graded metal application (Table 2). In the control plants, Ni concentrations were minimal, with 0.18 mg kg^{-1} in shoots and 0.08 mg kg^{-1} in flowers. The maximum Ni accumulation was recorded under the combined treatment of Ni @ 2 mg L^{-1} + Zn @ 10 mg L^{-1} (T8), reaching 10.20 mg kg^{-1} in shoots and 7.24 mg kg^{-1} in flowers. A similar trend was observed for Zn, with control (T1) plants containing only 0.60 mg kg^{-1} in shoots and 0.25 mg kg^{-1} in flowers, whereas the combined treatment (T8) resulted in the highest Zn concentrations of 62.77 and 43.70 mg kg^{-1} in shoots and flowers, respectively. The pronounced increase in metal accumulation under the combined treatment suggests a synergistic interaction, wherein the presence of one metal may enhance root membrane permeability or activate shared metal transport pathways, thereby facilitating greater uptake of the other. Comparable synergistic uptake patterns were reported by Al-Huqail *et al.* (2023), who observed preferential translocation of heavy metals into marigold shoots relative to floral tissues.

Table 2: Effect of application of graded concentration of Ni and Zn through irrigation water in metal content (Ni, Zn) of shoot, flower of marigold.

TREATMENT		Marigold			
		Shoot (mg kg^{-1})		Flower (mg kg^{-1})	
		Ni	Zn	Ni	Zn
Control	T1	0.18^{e}	0.60^{e}	0.08^{e}	0.25^{e}
Ni @0.2 mg/L	T2	3.68^{d}	0.66^{e}	1.89^{d}	0.29^{e}
Ni @1 mg/L	T3	4.57^{c}	0.74^{e}	2.66^{c}	0.32^{e}
Ni @2mg/L	T4	9.22^{b}	0.85^{e}	6.14^{b}	0.37^{e}
Zn @2mg/L	T5	0.18^{e}	14.40^{d}	0.09^{e}	6.75^{d}
Zn @5mg/L	T6	0.19^{e}	32.40^{c}	0.09^{e}	20.40^{c}
Zn @10mg/L	T7	0.22^{e}	58.40^{b}	0.09^{e}	41.20^{b}
Ni @2 mg/L +Zn @10mg/L	T8	10.20^{a}	62.77^{a}	7.24^{a}	43.70^{a}
CD (0.05)		**0.76**	**2.73**	**0.33**	**1.60**

Biomass yield of marigold shoot and flower

Biomass production in marigold exhibited clear variations in response to graded levels of nickel and zinc (Table 3). In the control treatment, shoot and flower biomass were 20.20 g pot^{-1} and 0.89 g pot^{-1}, respectively. A slight improvement was observed with low-dose Ni application (T2: 0.2 mg L^{-1}), where shoot biomass increased to 21.30 g and flower biomass to 0.93 g pot^{-1}, indicating that trace amounts of Ni may enhance physiological activity through its role in enzymatic functioning (Bosiacki & Wojciechowska, 2012). However, higher Ni levels reduced biomass accumulation, as seen in T4, which produced only 0.77 g of flower biomass. This decline aligns with previous observations that excessive Ni induces oxidative stress and reduces biomass in ornamentals (Verma *et al.*, 2022). A similar trend was observed under zinc treatments. The Zn @ 2 mg L^{-1} treatment (T5) generated the highest shoot biomass (21.53 g pot^{-1}) and stable flower biomass (0.92 g pot^{-1}), suggesting that moderate Zn enhances growth due to its involvement in auxin metabolism and enzyme activation. Increasing Zn concentrations to 5 and 10 mg L^{-1} (T6 and T7) led to reduced shoot and flower biomass, supporting earlier findings that Zn toxicity interferes with membrane stability and nutrient uptake (Al-Huqail *et al.*, 2023).

The combined application of Ni @ 2 mg L^{-1} + Zn @ 10 mg L^{-1} (T8) resulted in the lowest shoot (19.40 g pot^{-1}) and flower (0.70 g pot^{-1}) biomass, indicating synergistic metal toxicity. Similar interactions between heavy metals have been reported, where concurrent exposure enhances cellular stress and decreases photosynthetic efficiency (Verma *et al.*, 2022; Al-Huqail *et al.*, 2023). Overall, the results show that low to moderate levels of individual metals may slightly improve biomass, whereas high or combined concentrations of Ni and Zn cause significant growth suppression**, c**onsistent with known dose-dependent toxicity responses in marigold.

Table 3: Effect of application of graded concentration of Ni and Zn through irrigation water in shoot, and flower biomass yield of marigold.

TREATMENT		Biomass yield (g/pot)	
		Shoot	Flower
Control	T1	20.20^{c}	0.89^{a}
Ni@0.2 mg/L	T2	21.30^{ab}	0.93^{a}
Ni@1 mg/L	T3	21.00^{ab}	0.84^{ab}
Ni@2mg/L	T4	20.57^{bc}	0.77^{bc}
Zn@2mg/L	T5	21.53^{a}	0.92^{a}
Zn@5mg/L	T6	21.17^{ab}	0.87^{a}
Zn@10mg/L	T7	20.80^{abc}	0.74^{c}
Ni@2 mg/L +Zn@10mg/L	T8	19.40^{d}	0.70^{c}
C.D (0.05)		**0.78**	**0.09**

Heavy metal uptake

The influence of irrigation water containing graded levels of nickel (Ni) and zinc (Zn) on their uptake by marigold shoots and flowers is presented in Table 4. In the Control (T1), uptake of Ni and Zn by shoots was minimal (0.072 g/pot and 0.22 g/pot, respectively), while flowers accumulated only trace amounts (0.004 g/pot Ni and 0.012 g/pot Zn). Application of Ni @ 0.2 mg/L (T2) markedly enhanced Ni uptake in both shoots (1.769 g/pot) and flowers (0.078 g/pot), whereas Zn uptake remained almost unchanged. A further increase in Ni concentration (T3 and T4) produced a proportional rise in Ni uptake, reaching a maximum of 4.746 g/pot in shoots and 0.190 g/pot in flowers at Ni @ 2 mg/L (T4), consistent with dose-dependent Ni accumulation reported by Bosiacki and Wojciechowska (2012).

In the Zn treatments, Zn uptake increased sharply with higher Zn concentrations: 6.21 g/pot at 2 mg/L (T5), 17.66 g/pot at 5 mg/L (T6), and 30.57 g/pot at 10 mg/L (T7). Flower Zn uptake exhibited a similar trend, with maximum Zn accumulation (1.214 g/pot) observed at Zn @ 10 mg/L (T7). Notably, the combined application of Ni @ 2 mg/L + Zn @ 10 mg/L (T8) resulted in the highest uptake of both metals 5.095 g/pot Ni and 30.74 g/pot Zn in shoots and 0.198 g/pot Ni and 1.218 g/pot Zn in flowers indicating synergistic enhancement of metal acquisition under dual metal stress.

Overall, Ni uptake increased progressively with increasing Ni concentration, while Zn uptake rose with higher Zn doses. The combined treatment (T8) was most effective in promoting metal accumulation, suggesting a synergistic interaction between Ni and Zn that facilitates greater translocation and sequestration within marigold tissues. These results align with previous findings that marigold actively accumulates heavy metals in both vegetative and reproductive parts under elevated metal availability (Bosiacki & Wojciechowska, 2012), highlighting its strong potential as a bioindicator and accumulator species.

Table 4: Effect of application of graded concentration of Ni and Zn through irrigation water on uptake (Ni, Zn) of shoot, flower of marigold.

TREATMENT		Marigold			
		Shoot (g/pot)		Flower (g/pot)	
		Ni	Zn	Ni	Zn
Control	T1	0.072[d]	0.22[d]	0.004[e]	0.012[d]
Ni @0.2 mg/L	T2	1.769[c]	0.27[d]	0.078[d]	0.014[d]
Ni @1 mg/L	T3	2.235[b]	0.27[d]	0.096[c]	0.016[d]
Ni @2mg/L	T4	4.746[a]	0.29[d]	0.190[b]	0.018[d]
Zn @2mg/L	T5	0.083[d]	6.21[c]	0.004[e]	0.310[c]
Zn @5mg/L	T6	0.078[d]	17.66[b]	0.004[e]	0.685[b]
Zn @10mg/L	T7	0.067[d]	30.57[a]	0.005[e]	1.214[a]
Ni @2 mg/L+Zn @10mg/L	T8	5.095[a]	30.74[a]	0.198[a]	1.218[a]
CD (0.05)		**0.400**	**1.61**	**0.018**	**0.051**

Conclusion

Graded Ni and Zn application significantly influenced soil properties, metal bioavailability, tissue accumulation, biomass production, and metal uptake in marigold. Moderate Zn improved growth, while higher Ni and Zn induced toxicity. The Ni+Zn combined treatment produced the highest extractable metals, maximum tissue concentration, and greatest uptake, clearly demonstrating marigold's capacity to accumulate heavy metals.

These findings underscore the relevance of marigold as a bioindicator and potential phytoremediation crop in metal-contaminated environments. The results also highlight the importance of judicious micronutrient management to prevent metal accumulation in ornamental cropping systems.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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