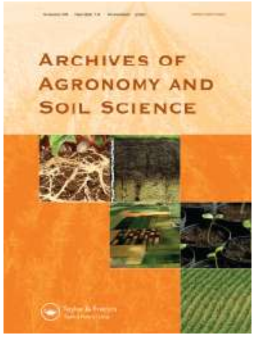

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Role of soil amendments on phytoremediation potential of marigolds on cadmium and zinc co-contaminated soil

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ABSTRACT

The influence of soil amendments (spent coffee grounds, bone meal, sewage sludge) on cadmium (Cd) and zinc (Zn) accumulation and growth performance of marigold (*Tagetes erecta* L.) varieties – Prime yellow, Twenty yellow and Dragon yellow – was evaluated. X-ray fluorescence was applied to determine the elements in tissues of cross-sectioned stems. Twenty yellow showed the highest percentage tolerance index values (151%) and percentage growth rate in dry biomass (164.2%) in Cd/Zn-amended treatments. The sewage sludge improved soil quality and promoted plant growth and also contributed to high Zn and Cd accumulation in Dragon yellow leaves (221.3 and 57 mg kg^{-1}, respectively). Dragon yellow variety exhibited potential for Cd phytoextraction potential as indicated by a bioconcentration factor for shoots >1 and translocation factor >1. In Dragon yellow grown in soil amended with BM, Cd and Zn were detected primarily in the epidermis and cortex. This may have resulted from the high P concentration in BM-supplemented soil, which increased co-precipitation of metals with P in epidermal cell walls. The enhancing properties of certain soil amendments can benefit marigold growers, as specific materials were found to support the growth of certain marigold varieties (for example, SS is considerably compatible with the Dragon yellow variety).

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Heavy metals; phytomanagement; *Tagetes erecta* L.; X-ray fluorescence

Introduction

Tailings ponds at a zinc mine in Mae Sot District, Tak Province, Thailand, are documented to have released fine sediments contaminated with excess quantities of heavy metals (HMs). This is particularly problematic during the rainy season, when overflow enters

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nearby streams that are used as irrigation water for agricultural plantations. Cadmium (Cd) and zinc (Zn) have been detected at substantial levels in soil and plants in the affected region; both metals have been demonstrated to bioaccumulate in food chains (Phaenark et al. 2024). Long-term consumption of Cd-enriched crops pose serious health impacts to humans as manifested by itai-itai (a form of proximal renal tubular dysfunction), malabsorption, anemia osteoporosis and osteomalacia (Somporn et al. 2023). Cadmium accumulation in crops, particularly Thai Jasmine rice (KDML105), has been linked to fatalities and illness among local villagers due to consumption of crops cultivated in contaminated areas (Saengwilai and Meeinkuirt 2021). Furthermore, crop productivity and quality can decline significantly as a result of the detrimental effects of Cd accumulation. Zinc (Zn) is a micronutrient required for growth and development of plants and animals; however, Zn becomes toxic when concentrations in living tissue surpass a critical level (Saleem et al. 2022). Several studies have reported on the interactions, i.e. adverse effects, of Cd and Zn in crop plants grown in the Mae Tao River basin in Mae Sot district, Tak province (Suwatvitayakorn et al. 2020). Few reports, however, have addressed the use of plants for phytomanagement of these metals with the goal of obtaining beneficial applications beyond edible uses. Several non-edible plants, i.e. energy crops and ornamental flowering plants, can be employed for phytomanagement of soil Cd; in such situations, degree of Cd removal is not the utmost priority. These plants are capable of tolerating severe environments; therefore, removing Cd in modest amounts is acceptable. Products derived from energy crops, e.g. *Jatropha curcas* L. (Jatropha) and *Acacia mangium* Willd (Acacia), can be used to generate revenue for local farmers and communities. *Jatropha* can grow in heavily Cd-contaminated locations and can be used for renewable energy or biodiesel; acacia produced substantial biomass, experienced low Cd accumulation with no toxicity symptoms and produced high lignin and cellulose content, making it suitable as a plant-based energy source (Taeprayoon et al. 2022).

Several flowering ornamental plants have been examined for tolerance to HM-contaminated soil while being monitored for growth performance and production of attractive blossoms. Two plant species, namely *Matricaria inodora* L. and *Achillea millefolium* L. of the Asteraceae family, demonstrated phytoextraction potential for manganese (Mn), copper (Cu), Zn, iron (Fe) and chromium (Cr). Both species accumulated HMs primarily in aboveground plant parts, particularly stems. Such findings attest that certain plant species grow and develop well in metalliferous soils due to marked tolerance to HMs (Glišić et al. 2021).

In several countries, marigolds (*Tagetes* spp.) are renowned for their iconic role in cultural ceremonies (e.g. religious worship); however, they offer the additional benefit of phytoremediation of HMs due to their ability for uptake combined with substantial biomass production and high rate of growth. Marigold roots possess unique traits that render tolerance to HM stress as well as allow for cultivation in HM-contaminated soil (Khilji et al. 2024). It has furthermore been demonstrated that fertilizers rich in essential macronutrients, i.e. nitrogen (N), phosphorus (P) and potassium (K), improve growth and enhance Cd accumulation in shoots of *Tagetes patula* L (Li et al. 2012; Haller et al. 2023).

Soil amendments (organic, inorganic-mineral and mineral types) are widely used immobilizing agents for contaminated soil; their ready availability and cost-effectiveness are key factors in support of sustainable remediation. Marigold cultivation in HM-contaminated soil is enhanced by use of soil amendments (Kaur et al. 2023). Recent research has demonstrated the effective usage of animal manure, vermicompost, biodigest and bone meal (van Midden et al. 2023). Among various amendments, sewage sludge has been less popular to date, partly as a result of restrictions to its application to soil (Garbowski et al. 2023). No investigation of the effects of sewage sludge on Thailand marigolds has been performed thus far. Similarly, little is known about the possible benefits of spent coffee grounds (SCGs), i.e. waste generated in the production of coffee products, on Thailand marigolds. This waste is a valuable source of biomass and various nutrients beneficial to sustainable development (Zhao et al. 2024).

It is generally accepted that phytomanagement can be implemented in numerous contaminated areas to mitigate the detrimental effects of HM contamination to biota and environmental quality while also providing a crop having commercial value and a source of income for the local population. Marigolds have been employed for phytoremediation of heavy metal contamination; however, it is crucial to determine which marigold variety can thrive in a specified harsh environment. Marigolds furthermore respond positively to various soil amendments, therefore it is important to determine which amendments enhance the nutrient supply for a designated marigold variety. The objectives of the current study are (1) to determine the effects of soil amendments on the growth performance of marigold varieties grown in Cd- and Zn-contaminated soil; (2) to determine the extent of Cd and Zn accumulation in marigold tissue; and 3) to determine elemental localization in specific tissues of test plants. It is hypothesized that soil amendments support the detoxification of HMs to marigolds and ultimately enhance growth and tolerance to metal stress.

Materials and methods

Soil sampling and preparation for analysis

Soil samples (0–15 cm depth) were collected during March 2021 from five locations (four corners and center) of a paddy field measuring approximately 7 ha in Pha De sub-district, Mae Sot District, Tak Province, Thailand (E 16° 670′ 38.600″ N 98° 620′ 63.1″), and contaminated with both Cd and Zn. The sampling area was bare soil, ready for cultivation with KDML105 rice cultivar. Relative humidity was reported at 69% using a hygrometer (RS Components Co., Ltd., Thailand), and the temperature was 29.4°C. Soil parent material is dark gray mudstone characterized by thin bedding, which is interlayered with gray to brown sandstone and limestone ranging from light gray to dark gray. Additionally, calcareous sandstone and limestone comprise some of the soil parent material over the Mae Tao River Basin region (Phomsenwong et al. 2019).

In the field, soil samples were stored in paper bags. In the laboratory, samples were oven-dried at 70ºC for 7 d, sieved (<2 mm) to remove gravel, stone and plant debris, and thoroughly mixed in order to obtain the composite soil. Four treatments were prepared as follows: contaminated soil (3.15 kg) amended with sewage sludge (0.35 kg or 10% w/w) (ConSS); contaminated soil (3.15 kg) amended with spent coffee grounds (0.35 kg or 10% w/w) (ConSCG); contaminated soil (3.15 kg) amended with bone meal (0.35 kg or 10% w/w) (ConBM); contaminated soil (3.5 kg) with no amendment (control) (NonCon or contaminated (Con) soil alone); and commercial soil (3.5 kg) with no amendment (NonCom or commercial (Com) soil alone).

Sewage sludge was obtained from the Nakhon Sawan municipal wastewater treatment system, Muang District, Nakhon Sawan Province. Domestic wastes comprise the source of the sludge. Spent coffee grounds were obtained from a commercial shop (Amazon) in Nakhon Sawan Province. Arabica and Robusta coffee were the species used in the experiments. The sludge and SCGs were allowed to dry at ambient temperature for 14 d, and then kept in the greenhouse prior to the experiments. Cattle bone meal was purchased from a commercial source in Nakhon Pathom Province, Thailand. Commercial soil was purchased from an agricultural supply shop in Nakhon Sawan Province. The commercial soil was a mixture of soil, bamboo seeds, food compost, animal manure and plant debris, prepared by the Takhian Luean Agricultural Group, Nakhon Sawan province, Thailand.

Determination of soil properties

Soil texture was determined by the hydrometer method (Allen et al. 1974). Soil pH was determined in a 1:5 soil:water (w/v) suspension using an Accumet AP115 pH meter, and electrical conductivity (EC) was determined using an EC meter (Hanna instruments; HI993310). Organic matter (OM) was determined by the Walkley – Black titration method (Walkley and Black 1934) and cation exchange capacity (CEC) by leaching with 1 M ammonia acetate at pH 7 (Ross et al. 2008). Water holding capacity (WHC), i.e. the quantity of water that a soil sample stores following equilibrium of saturation was ascertained using the method described by Alaboudi et al. (2018). Total N was determined by the Kjeldahl method (Black 1965), available (avail.) P by the Bray II method (Bray and Kurtz 1945) and avail. K by atomic absorption spectrophotometry after extraction with NH_4OAC (Warncke and Brown 1998). Soils were extracted with 0.005 M diethylenetriamine pentaacetic acid (DTPA) at pH 7.6 for 15 min and analyzed for Ca, Mg, Cd and Zn concentrations using inductively coupled plasma-mass spectrometry (ICP-MS; NelON2000; Perkin Elmer, U.S.A.). One-half gram of soil was microwave-digested (ETHOS One; Milestone Inc.) in conc. 70% nitric acid (HNO_3) and 30% H_2O_2, following which total Cd and Zn concentrations were determined using ICP-MS (Sparks et al. 1996).

Preparation of Cd and Zn stock solutions for irrigating soil

High purity analytical grade standard reagents served as Cd and Zn stock solutions. All solutions were prepared using deionized (DI) water and analytical grade reagents. A stock solution containing 0.064 mg Cd L^{-1} and 0.047 mg Zn L^{-1} was used to irrigate soil. The irrigation solution was prepared to simulate Cd and Zn concentrations measured in a contaminated stream in the Mae Tao River Basin in Tak Province, Thailand.

Greenhouse experiments

Seeds of three varieties of marigolds – Dragon yellow, Twenty yellow and Prime yellow – were purchased from AGA Agro Co. Ltd., Chiang Mai Province, Thailand. These varieties are popular by virtue of high biomass production, tolerance to tropical climates and preferred flower color and size for commercial use. Additionally, these varieties can be grown in a variety of soil types including those contaminated with HMs (Personal communication, AGA Agro Co. Ltd., Chiang Mai Province, Thailand).

Seeds were sterilized in 10% sodium hypochlorite (NaOCl) for 5 min, rinsed several times with tap water and followed by several rinsings in DI water. In a 1 L square plastic container (16 cm × 14 cm × 5.5 cm), seeds were cultivated in peat moss soaked in DI water for 7 d. A single healthy seedling was transplanted into a separate 3.5-L plastic pot that contained 3.5 kg soil in the greenhouse at Mahidol University, Nakhonsawan campus. All pots were transferred to the greenhouse and arranged in a randomized complete block design with five replicates on aluminum potting benches. Growing conditions in the greenhouse were as follows: temperature 27–32°C; relative humidity 65–85%; and light intensity 5,000–16,000 lx.

Soil moisture in each pot was maintained at approximately 70% water holding capacity (WHC) by addition of DI water and checked by weighing the pots twice per week (Alaboudi et al. 2018). Marigolds were cultivated over 3 months (November 2021 to February 2022). Every 15 d, 3 g of Osmocote fertilizer (16–16–16) was added to each pot to maintain nutrient levels. A total of 108.9 mL of stock solution was irrigated into pots twice per day (Chunwichit et al. 2024). The NonCom treatment received DI water only. Following harvest, flower diameter and number of flowers were measured; each plant part was then oven-dried and weighed to estimate percentage growth rate:

$$\text{Percentage growth rate in dry biomass} = (DBP_a - DBP_i)/\Delta t \times 100$$

where DBP_a is dry biomass after plant harvest, DBP_i is dry biomass before planting, and Δt is the time interval before harvest.

Heavy metal analysis

Marigolds were harvested after 90 d growth. Plants were carefully removed from pots, gently rinsed several times with tap water to remove attached soil, then rinsed three times with DI water. The above-ground (i.e. stems, leaves and flowers) and below-ground (i.e. roots) portions were separated. Tissue was oven-dried at 70°C for 5 d. Dried tissue was ground in a mortar and pestle (IKA; A11 basic), sieved through a 1-mm mesh sieve and weighed. One half-gram of dried plant material was placed in a vessel tube and digested on dry block heaters (IKA Works, Thailand) using 37% hydrochloric acid (HCl) and 70% HNO_3. Solutions were allowed to cool, then filtered (Whatman No. 42), and made to 25 mL volume with DI water. The digested solution was analyzed for Cd and Zn using ICP-MS (Sparks et al. 1996).

To determine soil Cd and Zn levels, 20 g of rhizosphere soil was removed from each pot. The sample was oven-dried at 80°C for 7 d, then ground to a fine powder with a mortar and pestle. The powder was sieved through a 60 mesh sieve (<0.25 mm). One-half gram of dried soil was digested with 70% HNO_3 and 37% HCl on dry block heaters at temperatures ranging from 60°C to 150°C. Cadmium and Zn concentrations were determined using ICP-MS (Sparks et al. 1996). For quality assurance and quality control, standard and blank solutions were analyzed before metal analysis. Furthermore, NIST 1515 apple leaves and 2711a Montana soil were used to calculate percentage recovery for plant and soil samples. Percentage recovery of Cd and Zn ranged from 93.4 ± 6.5% to 109.4 ± 25.2% and 95.2 ± 8.1% to 109.3 ± 17.8%, respectively.

Elemental localization in marigold stem tissues

X-ray fluorescence (XRF) is a non-destructive analytical technique that was used to determine element localization on a cross-section of marigold stem (1 mm diameter). Plant cuttings were selected from stem tissue that had been cut at 27.2 ± 2 cm from the base of the root, or at the middle of the stem. Plant tissue was oven-dried for 7 d at 40°C before being placed on a type-affixed acrylic (Figure S1) and analyzed using an X-ray fluorescence analytical microscope (XRF; HORIBA Scientific, Indonesia). One replicate is represented by five locations from each plant stem tissue; hence, stem tissues from five plants comprise five replicates. Localization of K, Ca, P, Fe, Zn and Cd was determined in selected tissues (cortex, epidermis, pith, phloem, sclerenchyma and xylem). X-ray tube operating conditions were 50 kV, 3932 s and 1000 μA and 30–50 kV, 100 s and 1000 μA.

Phyto-tolerance indicators

Bioconcentration factor (BCF)

The BCF is a ratio of metal concentration in shoot (C_{shoot}) to metal concentration in soil (C_{soil}). BCF values <1 indicate an excluder potential of HM; 1–10 suggest an accumulator of HM; and >10 indicates hyperaccumulation of HM. HM concentrations in plant organs and soil are expressed in mg kg^{-1} (Hussain et al. 2022).

$$BCF = C_{shoot}/C_{Ext.HM\ in\ soil}$$

Translocation factor (TF)

The TF index refers to the ability of a plant to transfer HMs from below-ground (C_{root}) to above-ground (C_{shoot}) organs. A TF value >1 indicates efficient HM translocation and greater HM accumulation in above-ground plant organs. The units of HM concentration in plant organs are expressed in mg kg^{-1} (Hussain et al. 2022).

$$TF = C_{shoot}/C_{root}$$

Tolerance index (TI)

Tolerance index (TI) is a measure of mean dry biomass production of the treated and control plants. A TI value >1 indicates that the plants tolerate the stress condition and produce greater biomass; TI values <1 indicate that plants are under stress and produce less biomass (Hussain et al. 2022).

$$\mathrm{TI} = \frac{\text{Biomass of the treated plants (g)}}{\text{Biomass of the control plants (g)}}$$

Statistical analysis

Data were evaluated using one-way ANOVA and post-hoc Least Significant Difference ($p < 0.05$; R-Package 'Agricolae' and 'ggplot2'). All data are expressed as mean ± SD. Principal component analysis (PCA), using the R-packages 'FactoMineR' and 'factoextra', was carried out to clarify the relationship between percentage elemental composition and selected tissues (cortex, epidermis, pith, phloem, sclerenchyma and xylem) of marigold species grown in the soil treatments. Analysis was conducted using R statistical software

Table 1. Physicochemical properties of soils used in this study (n = 5).

		Soil				
Parameter	Unit	NonCon	NonCom	ConSCG	ConBM	ConSS
Texture		Loam	Loam	Sandy clay loam	Sandy clay loam	Loam
Sand	%	45.0	44.8	45.0	45.0	45.0
Silt	%	29.6	37.6	27.6	27.6	29.6
Clay	%	25.4	17.6	27.4	27.4	25.4
pH		7.5	5.9	6.9	7.5	7.3
EC	dS m^{-1}	0.4	1.5	0.5	0.5	0.6
OM	%	2.4	6.8	6.5	3.0	3.0
CEC	cmol kg^{-1}	8.7	13.4	10.0	8.6	10.4
WHC	%	35.4	121.8	42.9	38.0	37.7
Total N	%	0.12	0.34	0.32	0.15	0.15
Avail. P	mg kg^{-1}	73.0	467.0	151.0	781.0	230.0
Avail. K	mg kg^{-1}	168.0	2749.0	229.0	162.0	195.0
Ext. Ca	mg kg^{-1}	4053.0	3378.0	3818.0	4096.0	4067.0
Ext. Mg	mg kg^{-1}	231.0	532.0	384.0	306.0	328.0
Total Cd	mg kg^{-1}	25.0 ± 3.2	0.8 ± 0.4	17.0 ± 1.0	22.2 ± 2.5	20.2 ± 0.4
Ext. Cd	mg kg^{-1}	15.6 ± 0.4	0.8 ± 0.1	12.1 ± 1.3	19.5 ± 0.9	17.6 ± 2.6
Total Zn	mg kg^{-1}	1,109.0 ± 107.4	54.7 ± 18.1	747.7 ± 52.2	1,022 ± 64.3	922.9 ± 23.1
Ext. Zn	mg kg^{-1}	810.5 ± 33.9	41.9 ± 5.7	632.7 ± 37.2	937.5 ± 47.1	873.7 ± 115.9

Con = Contaminated soil, Non = Non-amended soil, Com = Commercial product, SCG = spent coffee grounds, BM = Bone meal, SS = Sewage sludge, EC = Electrical conductivity, OM = Organic matter, CEC = Cation exchange capacity, WHC = Water holding capacity, *N* = Nitrogen, Avail. P = Available phosphorus, Avail. K = Available potassium, Ext. Ca = Extractable calcium, Ext. Mg = Extractable magnesium, Cd = cadmium, Zn = Zinc.

version 2.15.1 (RStudio-Team 2015). R-packages 'ggplot2', 'tidyr', and 'ggsignif' were used to construct the boxplots in different significant levels. SRplot online platform (Tang et al. 2023) was used to generate Pearson's correlation coefficients.

Results and discussion

Physicochemical properties of soils

Texture of NonCon, NonCom and ConSS soils was loam, whereas the ConSCG and ConBM were sandy clay loam (Table 1). All soil pH readings increased (to 6.9–7.5) following amendment application, and pH of the NonCom remained acidic (5.9). Values of EC, OM, CEC, WHC, total N and avail. K were all within tolerable ranges in the NonCom soil, where as avail. P and ext. Ca were high in the ConBM soil. In the amended and NonCon soils, concentrations of EC, OM, WHC, total N, avail. P and ext. Mg were lower by 1.3–3.8×, 1.3–2.8×, 1.1–3.4×, 1.3–2.8×, 2.1–10.7× and 1.3–2.3×, respectively, compared to the NonCom (control) soil. Substantial concentrations of total Cd and Zn were measured in the NonCon soil (25 and 1,109 mg kg^{-1}, respectively). The NonCom soil had lower total Cd and Zn concentrations (0.8 mg Cd kg^{-1} and 54.7 mg Zn kg^{-1}), and ext. Cd and Zn concentrations were 0.8 mg and 41.9 mg kg^{-1}, respectively.

Growth performance and tolerance index

The commercial soil treatment (NonCom) experienced the highest dry biomass growth rate (140–277%; Table 2 and Figure S2). This growth is the result of an abundance of essential nutrients and other growth factors to support development across all marigold varieties due to the absence of toxic metals. Robust growth is also reflected in the substantial dry weight of stems and in flower production. Dry biomass growth rate among the three varieties in the NonCom treatment is ranked as follows: Prime yellow (P) > Twenty yellow (T) > Dragon yellow (D). The NonCom treatment exhibited the greatest TI (180–340%; $p < 0.05$) compared to the other treatments. This soil treatment had very low concentrations of both Cd and Zn, thus benefitting the plants, unlike those grown in soil dosed with the Cd/Zn solution. Additionally, the commercial soil is a carefully prepared compost having a substantial SOM content (6.8%) and high water holding capacity (121.8%) and provides sufficient essential macro- and micronutrients to support optimum plant growth (Kumar et al. 2024). Despite the approximately similar OM content (6.5%) of ConSCG compared to NonCom (6.8%), the relatively poor plant growth of the former treatment may be due to the adverse effects of metals but also to WHC (42.9%) (Hussain et al. 2022).

In all amended treatments, only the Twenty yellow variety exhibited substantial percentage TI values, thus illustrating metal uptake in all tested plant parts (flower, stem, leaf and root), flower numbers and flower size, which are all relatively greater than those of the other varieties using the same amendments (Figure 1 and Table 2). These findings are consistent with previous reports that different marigold varieties have unique response abilities (morphological and floral attributes) to soils having different ratios of nutrients (natural or amended) and minerals (Taropi et al. 2022; Kaur et al. 2023).

Table 2. Growth performance of marigold varieties cultivated in the greenhouse (n = 5).

	Dry biomass (g)							
Treatment	Flower	Stem	Leaf	Root	Growth rate in dry biomass (%)	TI (%)	Flower diameter (cm)	Flower number
PNonCon	8.6 ± 0.6cdE	11.9 ± 1.2bE	8.1 ± 1.2bGH	7.4 ± 1.2dDE	100.0 ± 0.0bC	100.0 ± 0.0dH	5.3 ± 0.3bEFG	21.7 ± 1.5dD
PNonCom	20.8 ± 1.3aB	23.2 ± 2.3aC	11.3 ± 2.4aEF	8.9 ± 1.1bcC	277.0 ± 59.5aA	183.4 ± 6.6aD	6.0 ± 0.2aCD	51.4 ± 3.1aA
PConSS	9.0 ± 0.9cE	12.5 ± 2bE	10.1 ± 1.2aEFG	9.0 ± 0.7bC	110.1 ± 35.1bC	115.9 ± 7.5cG	5.3 ± 0.3bcFG	33.8 ± 3.4bB
PConBM	11.9 ± 0.5bD	11.5 ± 1.9bEF	9.7 ± 1.6abEFGH	11.7 ± 0.8aB	117.8 ± 53.1bC	127.8 ± 11.3bFG	4.8 ± 0.2dH	25.4 ± 1.1cC
PConSCG	7.6 ± 0.5dEF	7.4 ± 1.3cG	4.9 ± 2 cI	7.2 ± 1.7cdCD	104.1 ± 38.2bC	77.7 ± 13.2eI	5.0 ± 0.4cdGH	27.0 ± 1.9cC
TNonCon	8.8 ± 0.7dE	15.0 ± 2.4cDE	12.7 ± 2.1cDE	6.7 ± 0.1FG	100.0 ± 0.0bC	100.0 ± 0.0dH	5.9 ± 0.2bDE	12.0 ± 1.1dFGH
TNonCom	31.5 ± 1.4aA	41.9 ± 3.1aA	20.9 ± 1.8aB	14.2 ± 2.9aA	229.1 ± 81.5aAB	278.2 ± 10.2aB	6.4 ± 0.3aBC	33.2 ± 1.5aB
TConSS	14.4 ± 2.9bC	22.5 ± 3.5bC	16.2 ± 1.5bC	5.7 ± 1.6bcDEF	157.5 ± 70.1abC	151 ± 15.3bE	5.8 ± 0.3bDE	17.8 ± 3.6cE
TConBM	11.5 ± 0.4 cD	20.5 ± 3.5bC	13.9 ± 2.5bcCD	4.8 ± 1.9cEFG	164.2 ± 78abBC	130.1 ± 15.5cF	5.8 ± 0.6bDE	14.4 ± 0.5dF
TConSCG	13 ± 0.9bcCD	21.8 ± 5bC	14.4 ± 2bcCD	7.7 ± 2bCD	159.4 ± 68.8abC	146 ± 18.7bcE	5.9 ± 0.5abCD	26.6 ± 1.8bC
DNonCon	6.5 ± 0.5cFG	8.8 ± 1.3cFG	9.4 ± 1.6cFGH	3.1 ± 0.9bcGH	100.0 ± 0.0aC	100.0 ± 0.0dH	7.3 ± 0.2aDE	10.8 ± 0.9dH
DNonCom	21.2 ± 1.1aB	28.3 ± 3.3aB	26.5 ± 3aA	12.7 ± 2.2aAB	140.7 ± 61.1aC	341.2 ± 11.1aA	6.7 ± 0.2bB	33.8 ± 1.8aB
DConSS	6.5 ± 0.3cFG	7.7 ± 1.3cG	7.3 ± 1.4cHI	1.7 ± 0.6cH	130.2 ± 44.8aC	88.7 ± 10.3cHI	5.5 ± 0.4dEF	11.6 ± 0.5cdGH
DConBM	5.5 ± 0.4cG	6.3 ± 1.2cG	8.0 ± 1.2cGH	3.9 ± 1.9bFG	129.1 ± 51.1aC	90.9 ± 9.4cHI	6.1 ± 0.4cCD	18.2 ± 1.3bE
DConSCG	13.9 ± 2.4bC	15.8 ± 2bD	14.6 ± 2.8bC	13.2 ± 1.7aAB	111.0 ± 48aC	221.1 ± 9.8bC	6.0 ± 0.2cCD	13.4 ± 2.3cFG

p = Prime yellow, *T* = Twenty yellow, D = Dragon yellow, Con = Contaminated soil, Non = Non-amended soil, Com = Commercial product, SCG = spent coffee grounds, BM = Bone meal, SS = Sewage sludge. Values followed by the same letter are not significantly different ($p > 0.05$). Small letters represent differences among growth performance of all treatments within the same species; capital letters represent differences among growth performance of all plant species and all treatments.

Furthermore, the tested marigold varieties demonstrated high productivity in terms of flower size and number.

No appreciable differences were noted between biomass and accumulated metals for each plant organ ($p > 0.05$), based on the amendment type (Table 2) including a partial negative correlation ($p > 0.05$) (Table S1). Dry biomass values, however, especially in Prime and Dragon yellow varieties in amended treatments, experienced a higher percentage growth rate than in the NonCon treatment. The amended treatments, notably for the Twenty yellow variety, demonstrated substantial percentage growth rate in dry biomass (157.5–164.2%; $p > 0.05$). This corroborates the contention that such soil amendments aid plant growth in harsh conditions such as those caused by HM contamination. The bone meal treatment (ConBM) has a high P content (781 mg kg^{-1}), which is essential for root development. Bone meal has been utilized as a source of Ca, P and N as well as other elements, e.g. K, Fe, Mg, Fe and Na (Atemni et al. 2023). Application of spent coffee grounds (ConSCG) improved soil physicochemical properties, particularly OM content (6.5%), total N (0.32%) and Ext. Mg (384 mg kg^{-1}). Owing to its high total N content, grounds are an excellent supplement for compost or as a soil amendment (Bomfim et al. 2023). Application of sewage sludge provided substantial amounts of macronutrients (N, P and K).

Flower diameters in the Cd treatments were often smaller than those in NonCom (non-Cd) treatments, especially in Prime yellow and Twenty yellow varieties (Figure 1 and Table 2). When barium (Ba), Cu and Cr were added to the growth media of *Conyza canadensis* (L.) Cronquist (Family Asteraceae), both above-ground biomass and flower numbers were reduced, ultimately inhibiting plant growth and development (Zapryanova and

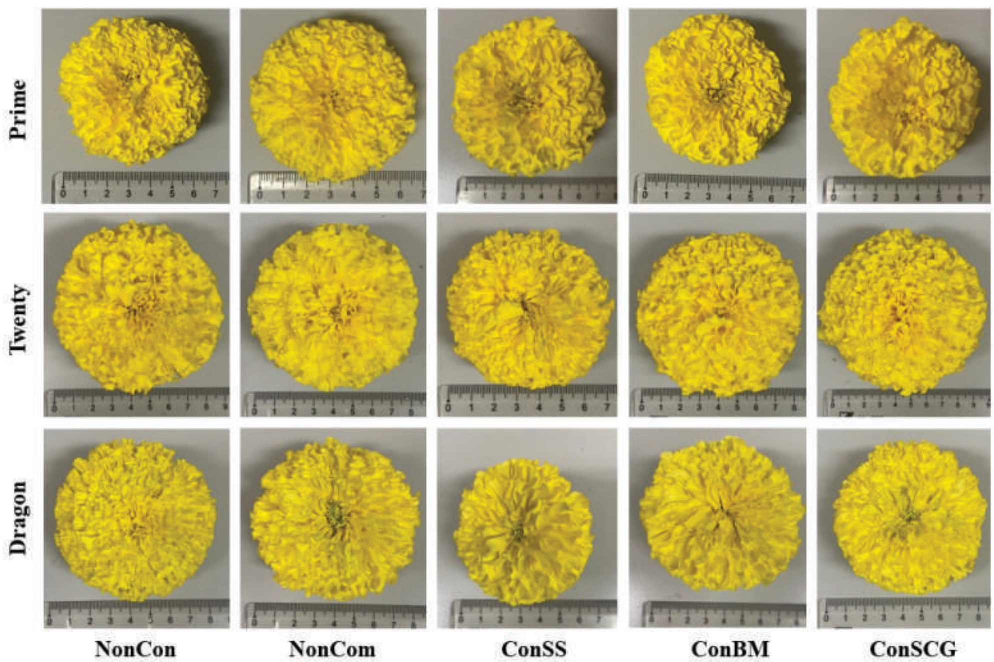

Figure 1. Marigold flower diameters (cm) as a function of variety and soil amendment. Abbreviations: Con, contaminated soil; Com, commercial soil; SS, sewage sludge; BM, bone meal; SCG, spent coffee grounds.

Atanassova 2009). According to Sun et al. (2018), different marigold cultivars are responsive to different Cd concentrations, particularly at high concentrations in contaminated soil. Cadmium also imparts adverse effects on marigold growth via disordering leaf anatomy (e.g. cell disorganization in vascular bundles, palisade and spongy mesophyll) (Thongchai et al. 2021). Dry biomass of flowers was significantly ($p < 0.05$) higher in the NonCom treatment as compared to all Cd/Zn soil treatments, which might result from a positive influence of Zn on marigold leaf and flower size (Table S1) due to its positive effects on foliar growth and development (Shah et al. 2016). The markedly higher flower numbers in all marigold varieties in the NonCom treatment reflect the overall high fertility and lack of toxicity. When compared to the Cd/Zn soil alone treatment, the NonCom treatment resulted in the highest flower numbers in the Dragon yellow variety (51.4, $p < 0.05$). Higher flower numbers were also measured in soil supplemented with sewage sludge, coffee grounds or bone meal (25.4–33.8). Sewage sludge as a soil improver increases flower numbers as documented elsewhere (Xue and Huang 2013; Al-Huqail et al. 2023). It is advisable to utilize these amendments as long-term soil inputs not only for optimal growth performance of marigold but also to enhance existing soil fertility (Solanki et al. 2016).

Heavy metal accumulation in plant tissue

Uptake of Cd in marigold tissue ranged from 2.7 to 57 in leaf, 1.6 to 20.2 in stem, 1.2 to 18.5 in root and 0.7 to 2.7 in flower, respectively; uptake of Zn ranged from 30.2 to 222.8 in

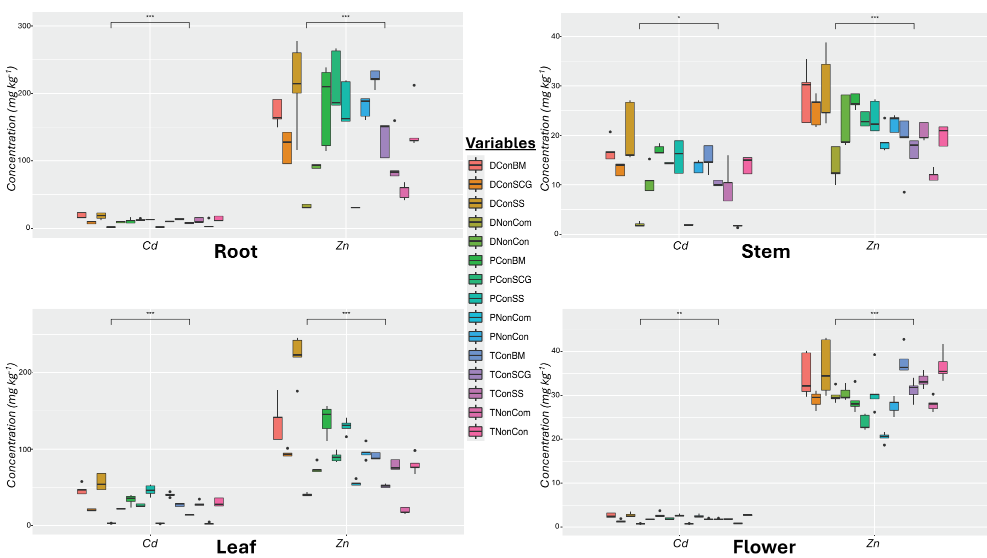

Figure 2. Box-plots displaying concentrations (mg kg^{-1}) of Zn and Cd in different plant organs based on marigold variety and soil amendment. Abbreviations: D, Dragon yellow; P, Prime yellow, T, Twenty yellow; Con, contaminated soil; Com, commercial soil; SS, sewage sludge; BM, bone meal; SCG, spent coffee ground. Asterisk (*) indicates the level of significance (* for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$).

root, 19.9–221.3 in leaf, 20.4–37.8 in flower and 11.8–28.9 in stem (Table S2). Overall patterns of Cd and Zn accumulation in plant tissue differ markedly; concentrations were in the following order: leaf > stem > root > flower; and root > leaf > flower > stem, respectively (Figure 2 and Table S2). Higher Zn uptake in roots than shoots, and higher Cd uptake in shoots than roots were reported in a previous study of marigold cultivation in metal-contaminated soils (Manadan et al. 2021); however, some marigold varieties, like Prime yellow in the NonCom treatment and Dragon yellow in NonCom and the ConSS treatments, had higher Zn concentrations in leaves than in roots. The Twenty yellow variety in the NonCom treatment had a higher Zn concentration in flowers than in leaves. The Dragon yellow variety for ConBM treatment had a higher Cd concentration in roots than in stems.

Plant species vary markedly in HM uptake depending on a suite of factors including plant physiology, soil HM concentration, HM bioavailability and soil chemical properties (Razmi et al. 2021). Many species of marigold have demonstrated potential for phytoremediation of soil contaminated with HMs (e.g. Cd, Cr, nickel (Ni)); different species illustrate varied metal uptake preferences by specific plant parts (e.g. stem, leaf, flower, root) (Saxena et al. 2022). In the present study, all tested parts of marigolds grown in commercial soil (NonCom) of all three varieties showed, as expected, the lowest content of Cd. In the case of Zn, however, only the root of all three varieties experienced the lowest Zn content, followed by the stem and leaf of Twenty and Dragon. Compared to other amendments, the ConSCG treatment was effective in increasing soil fertility by enriching OM content (6.5%) and lowering Zn and Cd concentrations (Table 1).

In both stem and leaf tissues, the highest concentrations (mg kg^{-1}) of both accumulated Cd (20.2 and 57) and Zn (28.9 and 221.3) were noted in the Dragon variety grown in SS-amended soil, followed by Prime and Dragon grown in BM-amended soil except in the case of Cd uptake in leaf (Figure 2 and Table S2). Despite low metal uptake (especially in flowers), Cd and Zn had accumulated in many tissues of marigold grown in SCG-, BM- and SS-amended soils (Table 1). Uptake and accumulation capabilities of Cd and Zn appear to be supported by these amendments (Biswal et al. 2021).

Highest Cd and Zn accumulation in roots was observed in BM-treated soils. Dragon root accumulated the most Cd (18.5 mg kg^{-1}) and Twenty the most Zn (222.8 mg kg^{-1}). Despite variable effects on marigold roots, the SS amendment supported root accumulation of Cd and Zn, particularly in Dragon (18.1 mg kg^{-1} for Cd and 213.6 mg kg^{-1} for Zn) and Prime (12.6 mg kg^{-1} for Cd and 182.9 mg kg^{-1} for Zn). Except for substantial Zn uptake by Prime variety (215.6 mg kg^{-1}), the Dragon and Twenty varieties experienced lower root metal uptake in SCG-treated soil. This may be a general behavior of horticultural plants, where it has been shown that SCG as amendment results in decline of growth, likely due to phytotoxic effects such as nitrate immobilization (Hardgrove and Livesley 2016).

Metal accumulation in flowers varied with marigold variety as well as with amendment. Highest accumulated Cd was in the ConSS treatment (2.71 mg kg^{-1}) for the Dragon variety and highest Zn in ConBM (37.82 mg kg^{-1}) for the Twenty variety, respectively. Heavy metal uptake of Twenty variety grown on non-amended soil was comparatively high (36.61 mg kg^{-1} of Zn and 2.68 mg kg^{-1} of Cd). Despite low metal uptake by flowers in amended soils, many marigolds still experienced greater metal uptake compared to those grown on unamended soil (contaminated and commercial soil). Cadmium application to soil can affect the pattern of Zn update in plants (Zhou et al. 2020). These metals may also interact to affect concentration in marigold petals (Moustakas et al. 2011). Amendment application will also affect Cd and Zn uptake and translocation. Different amendments had a positive and significant impact on morphological and floral attributes of marigolds (Abhishek et al. 2024).

Several studies have reported the phytoremediation potential of marigolds in metal-contaminated soils, including improved uptake performance in association with organic and inorganic soil amendments (Thongchai et al. 2021; Chunwichit et al. 2024). In the current study, amendment application had no discernible impact on soil pH (6.9–7.5). Despite the fact that soil pH of the ConSCG treatment was lowest (pH 6.5), it contains a tannin-embedding material that provides polyhydroxy polyphenol functional groups for the effective adsorption of HMs, which in turn reduces HM mobility (Kim and Kim 2020). This may explain why the SCG amendment offered potential for phytoremediation of HMs in soil in a study by Kim et al. (2014). Bone meal is a significant source of P and promotes plant growth and development (Majee et al. 2020). Addition of bone meal to contaminated soil may increase soil pH and lower HM availability via the formation of highly insoluble metal phosphates. Metal immobilization in soil may enhance the efficiency of phytostabilization, as HMs precipitate in rhizosphere soil and/or accumulate in roots (Taeprayoon et al. 2022). In the current study, a descending order of Zn accumulation in roots of all marigolds was observed by different soil amendments (222.8 mg kg^{-1} in Twenty of ConBM, 215.6 mg kg^{-1} in Prime of ConSCG and 213.61 mg kg^{-1} in Dragon of ConSS), indicating that metal uptake varies with plant species (Razmi et al. 2021).

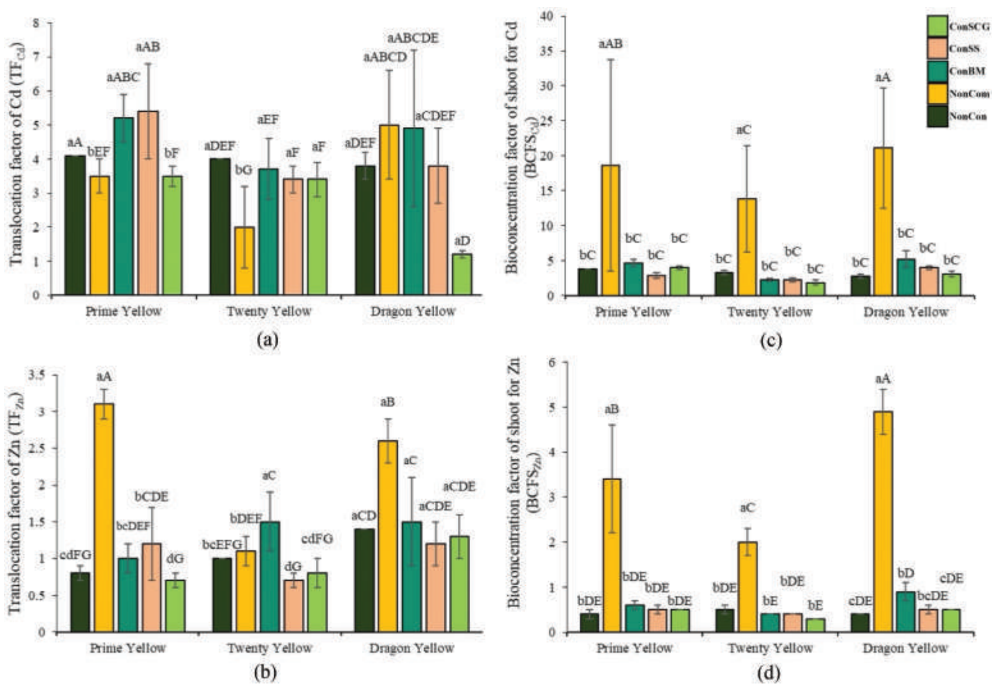

Figure 3. Accumulation index (a) Bioaccumulation factor of shoot for Cd (BCFS$_{Cd}$); (b) Bioaccumulation factor of shoot for Zn (BCFS$_{Zn}$); (c) Translocation factor of Cd (TF$_{Cd}$); and (d) Translocation factor of Zn (TF$_{Zn}$). Small letters represent differences among HM accumulation or translocation in each plant part in all treatments within the same species; capital letters represent differences among HM accumulation or translocation in each plant part in all plant species and all treatments.

Additionally, the growth of marigold is responsive to different soil inputs (*e.g.*, organic and inorganic fertilizers) (Majee et al. 2020). Based on greenhouse tests, the reported results indicate that marigold-based phytoremediation of Zn-contaminated soil must be based upon obtaining suitable marigold varieties. Soil amendments must also be thoroughly investigated for the potential to stimulate plant development, their capacity for absorption and accumulation and for offering tolerance to plants. The tested plants were unable to demonstrate hyperaccumulation, as demonstrated by relatively low Zn accumulation in shoots (<10,000 mg kg^{-1}; Reeves 2024), and BCFS values <1 for Zn and TF values >1 for Zn (Figure 3). Marigold varieties American, French, Babuda, Honey and Sunshine have been reported to have excluder potential, as they accumulate Cd and Zn primarily in roots (Thongchai et al. 2019). Additionally, bone meal can be used as a phosphorus fertilizer material; P-enriched amendments also immobilize heavy metals (HMs) including Cd and Zn, which in turn serves to remediate contaminated soils (Chen et al. 2024). SCGs are rarely applied as an amendment for phytoremediation of contaminated soil, due to the presence of insoluble toxic compounds (e.g. phenols) which may adversely affect the health of biota (Afriliana et al. 2021). In this study, a markedly low growth percentage was observed in all tested marigolds except Twenty grown in SCG-amended soil. Such poor response to SCG is consistent with a previous report that showed horticultural crops (broccoli, leek, radish, viola and sunflower) grew poorly in SCG-treated soil (Hardgrove and Livesley 2016).

Leaves of Dragon yellow in the ConSS treatment had the greatest Cd and Zn accumulation (57 and 221.3 mg kg^{-1}, respectively). Contaminated soil amended with SS did not significantly affect the growth and development of certain plants (Jatar; *Rosa multiflora* Thunb. ex Murray, and Virginia fanpetals; *Sida hermaphrodita* (L.) Rusby), and significant quantities of HMs accumulated in above-ground tissue, indicating the potential for hyperaccumulation (Bartlová et al. 2010). Metal uptake and accumulation patterns varied with the marigold plant part (Biswal et al. 2021), which might explain the somewhat greater Cd translocation to above-ground tissues compared to Zn in the current study. Consideration must be given to the optimum amendment application rate and other relevant variables in order to achieve the greatest phytoextraction potential (Deng et al. 2015). $BCFS_{Cd}$ values were >1 in all marigold varieties across all treatments (Figure 3). Furthermore, $BCFS_{Cd}$ values in all marigold varieties in the NonCom treatment were >10, indicating the plant is a hyperaccumulator of Cd (Lam et al. 2023). Since TF > 1, the TF_{Cd} supports the contention that Cd can be translocated from roots to shoots in all marigold varieties (Figure 3).

Localization of major elements in plant tissue

Localization of the elements K, Ca, P, Fe, Zn and Cd was observed in six specific stem tissues (Figure S1) using XRF analysis; accumulated concentrations were determined in light of marigold variety and amendment treatment (Figure 4; Figure S1 and Table S3). In PCA (Figure S3), the first two major components, i.e. 83.3% and 12.4% for Prime yellow; 72.9 and 21.7% for Twenty yellow; and 77.6% and 18.8% for Dragon yellow, were calculated and applied for display. Based on treatment conditions, K concentration was highly associated with several stem tissues, *e.g.*, xylem and phloem for all marigold varieties, and epidermis, cortex and phloem for Twenty yellow and Dragon yellow in the NonCom treatment. This treatment had the highest K concentration (2,749 mg kg^{-1}). Potassium concentrations tended

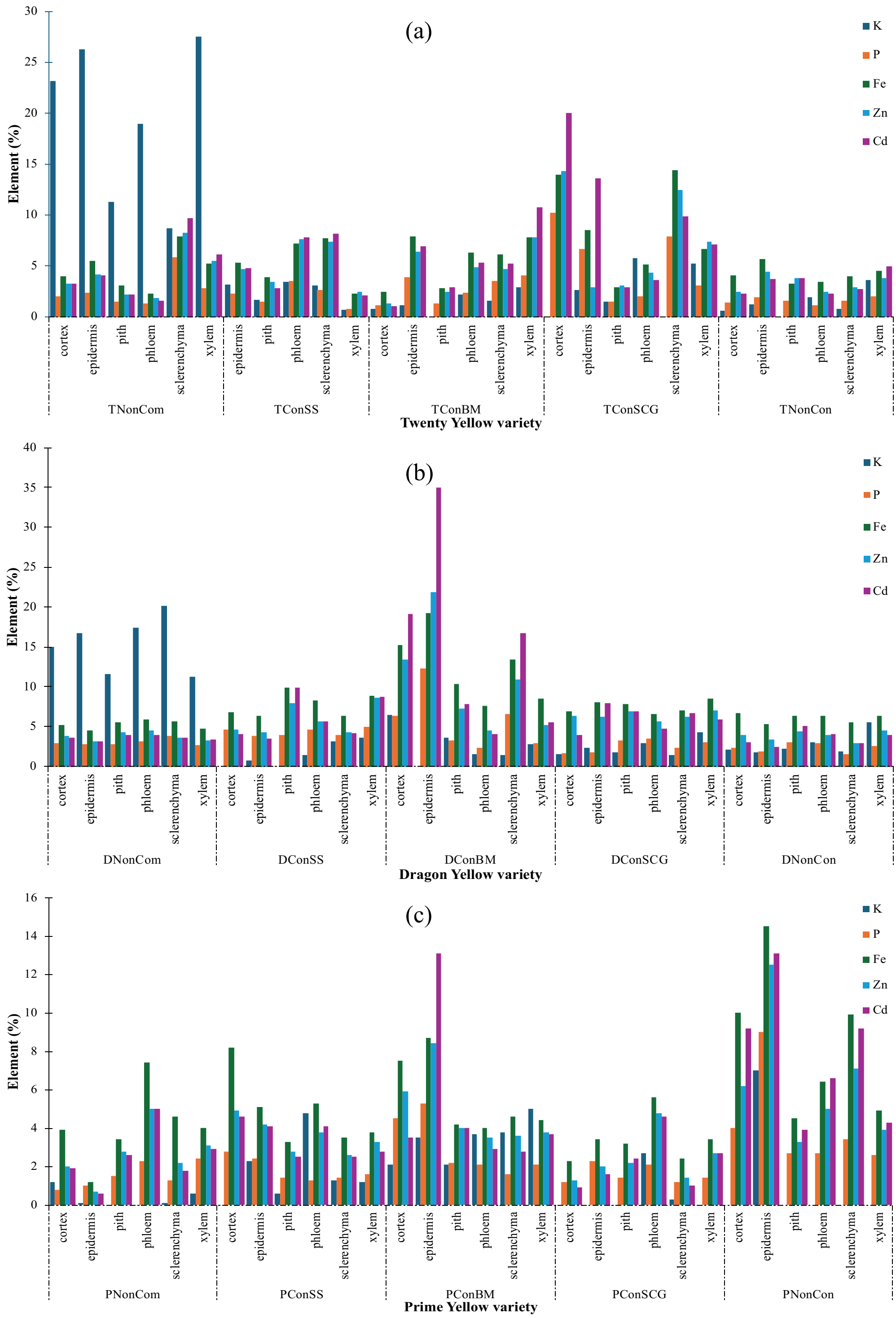

Figure 4. Elemental accumulation in specific tissue in cross-sectional stems from three marigold varieties as detected by XRF: (a) Twenty; (b) Prime; and (c) Dragon; grown in amended soil, contaminated soil and commercial soil. Abbreviations: Con, contaminated soil; Com, commercial soil; SS, sewage sludge; BM, bone meal; SCG, spent coffee grounds.

to be substantial in the surface tissue of marigolds. The surface layer, i.e. the epidermis, and the cortex immediately beneath are composed of thin-walled parenchymatous cells (Lisa et al. 2020). These cells also occur in the xylem (Tupan and Azrianingsih 2016). Calcium detected on the surfaces of stem tissue was associated with specific soil treatments; concentrations can increase significantly via the addition of organic fertilizer and other soil amendments (Ngui et al. 2024). Percentage Ca concentrations detected in different stem tissues, e.g. epidermis, sclerenchyma and cortex, were associated with NonCom and ConSCG treatments for Prime yellow variety; pith and cortex with NonCom and ConBM for Twenty yellow variety; and sclerenchyma with NonCom for Dragon yellow variety, respectively. Percentage Ca concentrations in stem tissue were generally higher as compared to other elements in stem tissue of all marigold varieties across all treatments (Table S1). Calcium has been considered a crucial second messenger for biological processes including physiology, immunity and overall plant growth (Singh and Pandey 2020). A cross-section of *Astragalus bisulcatus* (Hook.) A. Gray stem, a hyperaccumulator of selenium (Se), was utilized for generating a μXRF map that showed the distribution of Ca primarily in the cortex and epidermis of stem tissue (Barillas et al. 2012).

The elements P, Zn, Cd and Fe showed a high association with cortex, xylem and sclerenchyma in the ConBM treatment for Prime yellow, Twenty yellow and Dragon yellow varieties, respectively (Figure S3 a–c). This supports the hypothesis that a high P concentration in soil supplemented with bone meal might support co-precipitation of metals with P in specific plant tissues, primarily in epidermal cell walls (Ye et al. 2021). Phosphorus derived from other sources such as spent coffee grounds and sewage sludge may aid in the build-up of HMs like Zn, Cd and Fe. The marigold varieties in this study accumulated Cd primarily in above-ground parts; therefore, X-ray fluorescence can aid in detection of the preferential distribution of elements, which aids understanding hyperaccumulation processes of Cd. According to current findings, stems accumulated less total Cd than leaves and roots in the marigold varieties; this pattern of HM accumulation was consistent with that for other marigold varieties from previous studies (Rungruang et al. 2011; Shi et al. 2022). Investigating plant morphology, anatomy and physiology is crucial to understanding how plants adapt to various growth conditions, including those enriched with HMs. Bhagwat et al. (2022) reported that Cd was among the metals most likely taken up by marigolds; however, phytoaccumulation of Zn by marigolds is also effective (Sharma and Mathur 2022). Related research, in which comparable marigold varieties were cultivated in Cd and Zn co-contaminated soils, revealed that soil amendments, particularly vermicompost and biodigestate, may boost carotenoid concentrations in petals. This implies that soil amendments improve plant tolerance, growth and quantities of bioactive substances in marigold (Chunwichit et al. 2024).

Root traits of phytoremediators in HM-contaminated locations have been investigated to understand uptake and accumulation mechanisms. Vetiver (*Chrysopogon zizanioides* (L.) Roberty) demonstrated relatively high Cd accumulation in roots, reflecting its excluder potential which can be employed for phytoremediation (Phusantisampan et al. 2016). The moderate root length of marigold (<30 cm) implies that this plant can absorb HMs significantly in surface soils, as HMs such as Cd tend to accumulate in high quantities on the soil surface (Meeinkuirt et al. 2019; Hernández-Díaz et al. 2021).

Several reports using XRF analysis indicate Cd localization in epidermal structures of leaves in many plant species; such localization was identified in cortex parenchyma cells, endodermis, parenchyma cells of the central cylinder, trichomes and xylem vessels in the hyperaccumulator *Arabidopsis* (Isaure et al. 2006). In the current study, Dragon Yellow grown in contaminated soil amended with bone meal showed substantial percentage Cd accumulation in the epidermis (>30%; Figure 4). This structure may serve to reduce toxicity effects of Cd and enhance plant tolerance in harsh conditions (Printarakul et al. 2022).

Across all Cd/Zn soil treatments, elemental localization in marigold stems showed the trend: Ca > Fe > Zn ≈ Cd > p ≈ K (Table S3). However, substantial percentage K concentrations were found in almost all stem tissues in the NonCom treatments, especially in the Twenty yellow and Dragon yellow varieties. Cadmium concentrations in stem tissues were marginally higher than Zn concentrations. These two elements were primarily present in the epidermis and cortex, as observed in the Dragon yellow and Twenty yellow varieties in soil amended with bone meal and spent coffee grounds, respectively. Under high Cd concentrations (1,000 µM) in media, stem epidermal cells were thicker and showed substantial multicellular glandular trichomes in comparison to less developed cortex cells, revealing a defense mechanism in response to stress (Pandey et al. 2022).

Conclusions

Marigolds possess amendment-specific traits that must be investigated to determine potential effects on their growth and production. The current study demonstrated the detrimental effects of Cd/Zn to marigolds; evidence is also provided for improved growth and reduced HM toxicity to marigolds grown in sewage sludge-amended soil. Bone meal, a high P source, limited HM mobility in soil, thus improving marigold growth – such HM immobilization may improve plant response via different mechanisms. Application of spent coffee ground imparted positive effects on marigold growth in contaminated conditions. Twenty yellow marigold was the most adaptable among marigold cultivars grown under Cd/Zn-enriched conditions as evidenced by its greatest percentage tolerance index value and percentage growth rate in dry biomass. However, all varieties have potential as crop alternatives in metal-contaminated soil as indicated by flower quality (e.g. Dragon variety for flower size and Twenty variety for flower number). The XRF technique allowed for rapid and precise determination of elements in specific plant tissue. The results support the association between marigold variety and soil amendment; in addition, localization of elements within a specific tissue is identified. Such findings help clarify the role of soil amendments and their interactions with HMs in hyperaccumulator plants. Additional study is yet required; however, the findings are useful for marigold growers as well as local residents in terms of HM phytomanagement of contaminated fields. A selected marigold variety that is suitable for cultivation on contaminated soil may not necessarily have the ability to accumulate HMs in above-ground tissue; however, it may possess other qualities that are beneficial to the surrounding community. For example, marigold flowers are suited as a product for income generation by the local community while replacing edible crops.

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