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Efficacy of soil amendments and moss mulch on growth, heavy metal accumulation and secondary metabolites of marigold (*Tagetes erecta* L.) cultivated in organic soil contaminated with Cd and Zn

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ABSTRACT

This study evaluated the functional performance of two marigold varieties (*Tagetes erecta* L.), Thongchalerm (TCL) and Aga072 (AGA), grown in manufactured organic soil under Cd and Zn co-contaminated conditions. The soil was treated with individual or combined applications of Biovis (B), vermicompost (V) and moss (M). Moss was applied as a live plant mulch covering 90%, 45%, and 30% of the soil surface in pots. Biovis and V notably enhanced soil-available phosphorus, potassium and cation exchange capacity (CEC). The highest relative growth rate was observed in BM-treated soil for TCL and in BVM-treated soil for AGA. In TCL, B-treated soil showed the highest accumulation of carotenoids, phenolics, flavonoids and antioxidant activities (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and 2,2-diphenyl-1-picrylhydrazyl), while the control (organic soil) also exhibited similarly high values, indicating that organic soil alone provides sufficient nutrients. For AGA, the highest levels of secondary metabolites were observed in BVM-treated soil. Both TCL and AGA varieties showed maximum ferric reducing antioxidant power in BM-treated soil and α-glucosidase activity in VM-treated soil, indicating that moss application positively affected the secondary metabolite composition in marigolds. Translocation factor (TF) and bioaccumulation factor (BCF) values exceeded 1 for Cd, demonstrating effective Cd accumulation. For Zn, most treatments showed TF > 1 but BCF < 1, suggesting efficient metal translocation with limited overall accumulation. Overall, the findings highlight the phytoremediation potential of both marigold varieties for Cd and Zn, demonstrating their ability to maintain plant growth and enhance secondary metabolite production under contaminated conditions.

1. Introduction

Marigolds, belonging to the Asteraceae family, are flowering plants offering a rich source of aesthetic appeal, practical value, and industrial utility. Marigolds are edible flowers valued for their high nutritional content, diverse bioactive compounds, and growing potential in nutraceutical and functional food applications (Sultana et al., 2025). Hegde et al. (2023) has reported that marigolds contain high levels of total flavonoids and exhibit strong free-radical-scavenging and metal-chelating activities. Marigold flower extracts are commercially used as a rich source of natural bio-colorants and polyphenolic compounds with notable functional properties. Research has extensively

Abbreviations: ABTS, 2,2′-azino-bis3-ethylbenzothiazoline-6-sulfonic acid; AGA, Aga072 marigold; Ava_K, available potassium; Ava_P, available phosphorus; B, Biovis; BCF, bioconcentration factor; BM, Biovis with moss; BV, Biovis with vermicompost; BVM, Biovis with vermicompost and moss; Cd, Cadmium; CEC, cation exchange capacity; CRD, complete randomized design; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EC, electrical conductivity; FRAP, ferric reducing antioxidant power; HM, heavy metal; ICP-MS, inductively coupled plasma mass spectrometry; M, moss; OM, organic matter; PCA, principal component analysis; RGR, relative growth rate; TCL, Thongchalerm marigold; TF, translocation factor; TFC, total flavonoid content; TPC, total phenolic content; V, vermicompost; VM, vermicompost with moss; WHC, water holding capacity; Zn, Zinc.

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characterized key metabolites and bioactivities in marigold flowers, including carotenoids, phenolics, flavonoids, tocopherols, fatty acids, and inhibitory activities against α-amylase and α-glucosidase (Parklak et al., 2023; Saini et al., 2023; Suwanklang et al., 2023).

Marigolds are also recognized for their phytoremediation potential for heavy metals (HMs), owing to their ability to tolerate and accumulate these contaminants while maintaining healthy growth in contaminated soils. As hyperaccumulator species, marigolds have been widely used for the remediation of HM-contaminated sites (Sharma and Mathur, 2023). However, their bioaccumulation ability may depend on the type of chelating agent applied. For instance, the composite chelator GAC6 (glycine:aspartic acid:cystine = 4:10:6) has been reported to increase bioavailable Cd by more than 70% compared with untreated soil, thereby enhancing phytoextraction potential (Cao et al., 2025). Conversely, soil amendments—particularly organic materials—is known to enhance soil physicochemical properties and promote HM immobilization, reducing phytoavailability and phytotoxicity (Thongchai et al., 2021). Therefore, the use of chelating agents and organic amendments may exert contrasting effects concerning metal mobility and plant uptake, depending on remediation objectives.

Various soil remediation techniques have been developed to mitigate the risks associated with HM accumulation in soils. Among these, soil improvement through amendment application is one of the most widely adopted approaches due to its cost-effectiveness and environmental sustainability (Lwin et al., 2018). Despite a gradual response of organic soil amendments, they enhance crop productivity while minimizing environmental risks. In particular, vermicompost is a widely used organic fertilizer that has been shown to improve soil fertility and support marigold cultivation (Blouin et al., 2019; Cruz et al., 2024; Makkar et al., 2023). Biogas digestate has also been reported as a potential soil amendment (Lee et al., 2021), contributing to crop productivity, particularly when combined with other organic manure (e.g., animal manure) or inorganic fertilizers, e.g., N, P, and K (Adekiya et al., 2025). Therefore, applying organic soil amendments, either individually or in combination, is beneficial for marigold cultivation due to their positive effects on plant morphology and floral characteristics.

In pot-based systems, maintaining adequate and stable soil moisture remains challenging, even under greenhouse conditions. Irrigation constraints, including drought and waterlogging, often limit optimal plant growth. Cover cropping is among promising agronomic practices to promote soil health and crop growth performances in sustainable agroecosystems (Quintarelli et al., 2022). Among soil cover plants, mosses have been reported as effective plant mulches buffering extremes in soil moisture and temperature, while enhancing nutrient retention from applied fertilizers (Dollery et al., 2022). Moreover, moss acts as an efficient biosorbent, capable of accumulating substantial amounts of HMs, thereby reducing metal bioavailability and toxicity in the root zone and limiting metal translocation from soil to plant tissues (Phaenark et al., 2024). Collectively, these attributes may alleviate metal-induced stress in co-cultivated plants.

Crop replacement with marigolds in HM-contaminated fields, combined with soil amendments, shows strong potential due to the synergistic effects of amendments applied during marigold cultivation. Comprehensive research attention remains broad, focusing on diverse marigolds, amendment, and contaminated materials. The present study investigated the efficacy of soil amendments (Biovis and vermicompost) and moss on the growth attributes of two marigold varieties, Thongchalerm (TCL) and Aga072 (AGA), cultivated in small-scale, manufactured organic soil, irrigated with Cd- and Zn-co-contaminated water. The main objectives of this study were to elucidate how marigold varieties respond to individual or combined applications of Biovis, vermicompost and moss, and to assess their effects on growth, phytoaccumulation potential, and plant biochemical composition under Cd and Zn-co-contaminated conditions. Specifically, the study aimed to: (1) determine the relative growth rate of tested marigold varieties based on accumulated dry biomass (2) assess their phytoremediation potential under Cd and Zn co-contaminated conditions and (3) evaluate the functional composition of flower extracts, including bioactive compounds, antioxidant capacity, and enzyme inhibitory activity, in response to different treated materials. It has been hypothesized that applying organic soil amendments and mosses would mitigate metal-induced stress in marigold varieties grown in Cd and Zn co-contamination to a manageable level; thereby, enhancing plant growth and functional performance aligned with environmental sustainability principles. Moreover, integrating organic soil with supplementary amendments would advance sustainable waste management, improve crop productivity with stable organic residues, and enhance soil quality through bioaccumulation-driven processes strengthening environmental sustainability.

2. Materials and methods

2.1. Greenhouse experiment and research materials

A greenhouse experiment was conducted at Mahidol University, Nakhon Sawan, Thailand (N15°34′48.1″ E100°08′50.3″). An artificially manufactured soil, hereafter referred to as ‘organic soil’, was used in this study, as recommended by Mahidol University, Agricultural and Environmental Utilization Research Unit. The soil was formulated by mixing paddy-field topsoil (0–20 cm), composted *Samanea* leaves, cow manure, chicken manure, chopped coconut husks, and blended rice husks. The materials were incorporated at a 65:10:10:10:5 (w/w) ratio. The organic soil was treated with two organic amendments and moss, hereafter referred to as the treated materials. The amendments included vermicompost and Biovis, sourced from their respective suppliers: vermicompost from a local farm in Nakhon Sawan Province and Biovis from Biosynthai Biotechnology Co., Ltd. Additionally, Christmas moss (*Vesicularia montagnei* (Bél.) Broth. was used as a treatment and applied as a living plant mulch to observe its interactions with the tested marigolds. Moss was purchased from a local gardening store (HOMDOI Moss Garden). Marigold seeds of two varieties, Thongchalerm (TCL) and Aga072 (AGA), were purchased from local vendors at the Pak Khlong Talat night market, Bangkok, Thailand.

The experiment was conducted using a completely randomized design with five replications. Eight treatments were tested, including non-treated organic soil and organic soil treated with one or a combination of treated materials (Table S1). A total of 2 kg of soil was placed in each 2-L plastic pot (surface area of 531 cm^2, specifically 26 cm top diameter, 17 cm bottom diameter and 20 cm height). For the moss treatment, live moss (∼ 1 cm thick) was placed on the soil surface immediately after transplanting marigold seedlings to the pots. Moss as a live plant mulch, as demonstrated in Fig. S1, was applied by area coverage (90, 45 and 30% of the soil in the pots). For the Biovis treatment, 0.75 mL of Biovis (gaseous solution) was added twice weekly until the first harvest of marigold flowers. Before the experiment, vermicompost was thoroughly mixed with organic soil at a rate of 15% w/w (0.3 kg). Water contaminated with both Cd (0.064 mg L^{-1}) and Zn (0.047 mg L^{-1}) was prepared to match concentrations reported in the Mae Tao River Basin, Tak Province (Chunwichit et al., 2025). According to plant growth, approximately 75 mL of Cd- and Zn-co-contaminated solution was applied to each pot twice daily during the first 40 days, and 200 mL was used for the remaining days until harvest. Preparing the seed tray, transplanting and basic agronomic practices were conducted as described in Chunwichit et al. (2025), except that the Osmocote fertilizer application rate we calculated was based on 2 kg soil basis, e.g., 1.7 g per pot per application time. Briefly, marigold seeds were sown in seed trays February 10, 2025 and the seedlings were transplanted February 20, 2025. The greenhouse conditions were maintained at average temperature of 29 °C, relative humidity of 68%, and light intensity of 10,000 lx.

2.2. Determining soil properties

Before the experiment, soil samples subjected to individual or combined applications, including non-treated organic soil (control), were analyzed for physicochemical properties following methods and protocols applied in Chunwichit et al. (2025). Briefly, soil texture was determined using the hydrometer method (Allen et al., 1974), organic matter (OM) content by the Walkley–Black dichromate oxidation method (Walkley and Black, 1934), soil pH employing an Accumet AP115 pH meter, electrical conductivity (EC) by a portable EC meter (Hanna Instruments, HI 993310), total N applying the Kjeldahl method (Sáez-Plaza et al., 2013), cation exchange capacity (CEC) by leaching the soil with 1 M ammonium acetate at pH 7 (Ross et al., 2008), available phosphorus (Ava_P) by the Bray II extraction method (Bray and Kurtz, 1945) and available potassium (Ava_K) by inductively coupled plasma mass spectrometry (ICP-MS, NexlON 2000; Perkin Elmer, USA) (APHA, AWWA, WEF, 2005). Extractable Ca and Mg contents were also determined using ICP-MS.

2.3. Determining growth performance attributes

Harvesting of fully bloomed marigold flowers commenced approximately two months after growth, April 19, 2025. Fresh weight and diameter of harvested flowers were measured and recorded, after which the flowers were dried in an incubator at 35 °C. The entire marigold plant was harvested after three months of growth (May 27, 2025), and samples were collected to evaluate growth performance parameters, including plant height, root length, and dry biomass (Chunwichit et al., 2025). Moss samples were removed from the treated pots before harvesting marigold plants for further analysis. Plant materials were first washed with tap water to remove attached soil particles and debris, then rinsed with deionized water (DI) to eliminate remaining impurities. The samples were blotted dry using lint-free tissue paper to remove excess surface moisture. Each marigold plant was separated into roots, stems, leaves, and flowers for subsequent analysis, including growth performance assessment (dry biomass), determining treated Cd and Zn concentrations, and extracting metabolic compounds. The relative growth rate (RGR) was calculated following the formula described in Pandey et al. (2017).

$$RGR = (\ln W_2 - \ln W_1) / (t_2 - t_1) \quad (1)$$

Where, W_1 = initial dry weight of plant at time t_1 and W_2 = final dry weight of plant at time t_2

2.4. Determining bioactive compound, antioxidant and inhibitory activity

2.4.1. Preparing sample extract

Flower samples were extracted following Ratananikom and Premprayoon (2022) with modifications. Briefly, 1 g of dry sample was extracted using 50% ethanol in an ultrasonic bath at 350 W and 50 Hz for 30 min. An aliquot of supernatant was collected after centrifuging at 12,000 rpm for 10 min.

2.4.2. Determining bioactive compound

Determining total phenolic content (TPC): The TPC was extracted using the Folin–Ciocalteu assay as described in Singleton et al. (1999). Briefly, 20 μL of crude extract was mixed with 500 μL of a 10% Folin–Ciocalteu reagent for 10 min, then kept in the dark for 60 min after adding 1 mL of 7.5% (w/v) sodium carbonate. Absorbance was measured at 765 nm, and TPC was estimated using a calibration curve in mg gallic acid equivalent (GAE) per gram dry weight.

Determining total flavonoid content (TFC): The TFC of each extract was measured following the aluminum chloride colorimetric technique (Wolfe et al., 2003). Briefly, 200 μL of crude extract was mixed with 150 μL of 5% (w/v) sodium nitrite and incubated at ambient temperature for 5 min. Then, 150 μL of 10% (w/v) aluminum chloride was added. After incubating 6 min, 500 μL of 1 N sodium hydroxide was added to the mixture. Total volume was adjusted to 1.5 mL with 500 μL of distilled water. Absorbance was evaluated at 510 nm. The TFC was expressed as mg Rutin equivalent (RU) per gram of dry weight by comparing the rutin standard curve.

Determining total carotenoid content: Total carotenoids were extracted following Lichtenthaler (1987) with modifications. Briefly, 0.2 g of dried marigold petals was extracted with 10 mL of 80% acetone, then filtered with Whatman no. 42 filter paper. Three mL of 80% acetone was added, and the mixture was centrifuged at 7825 × g for 5 min. The supernatant was transferred to a cuvette, and the absorbance was measured at 663 nm (chlorophyll *a*), 646 nm (chlorophyll *b*), and 470 nm (carotenoids) using a UV–visible spectrophotometer. Pigment coloration was calculated as follows:

$$\text{Chlorophyll } a\ (\text{mg mL}^{-1}) = 12.21\ (A_{663}) - 2.81\ (A_{646}) \quad (2)$$

$$\text{Chlorophyll } b\ (\text{mg mL}^{-1}) = 20.13\ (A_{646}) - 5.03\ (A_{663}) \quad (3)$$

$$\text{Total chlorophyll}\ (\text{mg mL}^{-1}) = \text{Chlorophyll } a + \text{Chlorophyll } b \quad (4)$$

$$\text{Total carotenoid}\ (\text{mg mL}^{-1}) = 1000\ (A_{470}) - 1.8\ (\text{Chl } a) - 85.02\ (\text{Chlorophyll } b) \quad (5)$$

2.4.3. Determining antioxidant activity

The antioxidant potential was evaluated in terms of radical-scavenging activity—including 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)—as well as reducing power, such as ferric-reducing antioxidant power (FRAP), using their respective standard methods with slight modifications.

DPPH radical scavenging assay: Antioxidant activity by DPPH was measured following the method of Brand-Williams et al. (1995). A mixture of 100 μL of each crude extract and 1.40 mL of a 0.1 mM DPPH solution was incubated in the dark at room temperature for 15 min. Trolox was used as a positive control, and absorbance was measured and recorded at 517 nm. The percentage of DPPH scavenging activity was computed using the equation:

$$\text{Scavenging activity } (\%) = (\text{Abs}_{control} - \text{Abs}_{sample}) / \text{Abs}_{control} \times 100 \quad (6)$$

ABTS radical cation scavenging assay: ABTS radical cation was generated using potassium persulfate (2.45 mM) and 7 mM ABTS solutions. A mixture of 100 μL of crude extract and 1.4 mL of ABTS solution was kept in the dark at ambient temperature for 15 min. Absorbance was measured at 734 nm (Re et al., 1999). The ABTS radical cation scavenging activity was reported as a percentage using Eq. (6).

FRAP Assay: Each extract was tested for FRAP according to Benzie and Strain (1999). The working solution was prepared with 300 mM acetate buffer adjusted to pH 3.6, 10 mM 2,4,6 tripyridyl-s-triazine (TPTZ) in 40 mM HCl, and 20 mM $FeCl_3$⊡6 H_2O in a ratio of 10:1:1. Next, 100 μL of each crude extract was mixed with 1.4 mL working solution and incubated in the dark for 15 min. Absorbance was measured at 593 nm. A calibration curve was generated using Trolox in μM Trolox Equivalents (TE) per gram of dry weight.

2.4.4. Determining α-glucosidase inhibitory effect

The inhibitory activity of α-glucosidase was determined using the technique described by Kim et al. (2005) with minor modifications. Fifty μL of crude extract was preincubated with 0.05 U/mL α-glucosidase in 0.1 M phosphate buffer, pH 6.8, for 10 min. Following preincubation, 1 mM *p*-nitrophenyl-α-D-glucopyranoside in 0.1 M phosphate buffer, pH 6.8, was introduced and incubated at 37°C for 30 min. To stop the reaction, 1 mL of 0.1 M sodium carbonate was added to the mixture. Absorbance was measured at 405 nm. The percentage of α-glucosidase inhibition was calculated using the equation:

$$\text{Percentage inhibition (\%)} = (Abs_{control} - Abs_{sample})/Abs_{control} \times 100 \quad (7)$$

2.5. Determining Cd and Zn

Cadmium and Zn concentrations were determined in both soil and plant parts (root, stem, leaf and flower) following the procedure described in Chunwichit et al. (2024). Briefly, approximately 0.5 g of each sample was digested with 70% HNO_3 and 30% H_2O_2 (for soil) or conc. HCl (37%, for the plant) using a microwave digestion system (ETHOS One; Milestone Inc.). The digested solution was analyzed for Cd and Zn using ICP-MS (APHA, AWWA, WEF, 2005). Analytical accuracy was verified using certified reference materials, including NIST@ SRM@ 1515 (apple leaves) for plant samples and NIST@ SRM@ 2711a (Montana II soil) for soil samples, with metal recoveries ranging from 90% to 110%. Percentage recovery values obtained for Cd and Zn ranged from 90.3 ± 10.3% to 110.4 ± 22.6% and from 92.3 ± 10.1% to 108.7 ± 14.8%, respectively.

2.6. BCF and TF

The BCF is a measure of metal concentration in the shoot (C_{shoot}) to metal concentration in soil (C_{soil}). BCF values < 1 indicate HM excluder potential; values of 1–10 suggest a HM accumulator; and values > 10 indicate HM hyperaccumulation. BCF was calculated via the formula:

$$BCF = C_{shoot}/C_{Ext.\ HM\ in\ soil} \quad (8)$$

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 to above-ground plant organs. TCF was calculated via the formula:

$$TF = C_{shoot}/C_{root} \quad (9)$$

2.7. Statistical analysis

All statistical analyses were performed using one-way analysis of variance (one-way ANOVA) followed by Tukey's post-hoc test in RStudio (Version 2025.09.0 +387) (Allaire, 2019), with the packages 'dplyr' and 'multicompView'. Mantel's test (Mantel, 1967) was conducted using the packages 'tidyverse' and 'linkET', and principal component analysis (PCA) was performed using the 'FactoMineR' and 'factoextra' packages in RStudio. The SRplot online platform (Tang et al., 2023) was used to generate Pearson's correlation coefficients (r) and corresponding p-values.

3. Results

3.1. Soil properties

Soil chemical and physical properties varied with treatment (Table 1); however, most differences were insignificant ($p > 0.05$). Soil pH ranged from 6.8 to 7.5, EC from 3.6 to 5.0 dS m^{-1}, OM content (%) from 18.6 to 21.4, Ca from 4042 to 4290 mg kg^{-1}, Mg from 1573 to 1799 mg kg^{-1}, and total N (%) from 0.9 to 1.1. Available phosphorus and Ava_K were significantly ($p < 0.05$) higher in the control (2091 and 7475 mg kg^{-1}, respectively) compared with amended soils, particularly in Biovis-treated soil, which had 1640 mg kg^{-1} Ava_P and 6265 mg kg^{-1} Ava_K. In addition, CEC and WHC varied considerably: both were lowest in vermicompost-treated soil (15.6 cmol kg^{-1} CEC and 34.9% WHC), while the highest values were in the control (25.6 cmol kg^{-1} CEC and 210.2% WHC).

3.2. Growth performance

No significant variation was observed in flower number or diameter across treatments. Significant plant responses to treated materials were observed in the dry biomass of stems, leaves, and roots for both marigold varieties (Fig. S2 and Table 2). The TCL variety grown in BM-treated soil had the highest dry biomass of stem (27.8 g), leaf (9.8 g), and root (4.4 g), while the AGA variety grown in BVM-treated soil produced the highest dry biomass of stem (15.5 g) and leaf (10.4 g). Root biomass was the greatest in the root (1.5 g) in BM-treated soil. Despite these differences, however, the RGR of the varieties under each treatment did not differ markedly. All treatments (except M and BVM in TCL; M in AGA) showed higher RGR compared with the NT (Table 2). No significant findings were noticed in the number and size (diameter) of flowers in both varieties. However, the number of flowers (9.0–21.3) for the TCL variety in each treatment was consistently higher than that of the AGA variety (5.3–10.3).

3.3. Bioactive compounds (TPC, TFC and carotenoids)

Significant variations were noted for TPC (57.0–91.9 mg GAE g^{-1}) and TFC (26.3–37.3 mg RU g^{-1}) in the TCL variety, and for TPC (67.9–90.0 mg GAE g^{-1}) and TFC (28.8–39.4 mg RU g^{-1}) in the AGA variety (Table 3). Specifically, in the TCL variety, both TPC (57.0 mg GAE g^{-1}) and TFC (26.3 mg RU g^{-1}) were the lowest in M-treated soil; however, the maximum TPC was observed in B-treated soil (91.9 mg GAE g^{-1}), while the maximum TFC was found in the control (37.3 mg RU g^{-1}). In the AGA variety, both average TPC (90.0 mg GAE g^{-1}) and TFC (39.4 mg RU g^{-1}) values were the highest in the BVM-treated soil,

Table 1
Soil properties affected by various soil amendments at the start of the experiment.

Parameter	Ctrl	Vermicompost (V)	BioVis (B)	V + B
pH	6.8 ± 1.1a	7.5 ± 1.7a	6.9 ± 0.7a	7.3 ± 0.4a
EC (dS m^{-1})	5 ± 2.2a	3.6 ± 2.6a	4.2 ± 2.4a	4.0 ± 2.6a
OM (%)	20.5 ± 1.9a	21.4 ± 1.4a	18.6 ± 1.6a	19.5 ± 1.2a
Available P (mg kg^{-1})	2091 ± 190.2a	2117 ± 171a	1640 ± 77.4b	1960 ± 250.5ab
Available K (mg kg^{-1})	7475 ± 132.8a	6875 ± 94b	6265 ± 87.9d	6660 ± 116.6c
Ca (mg kg^{-1})	4250 ± 213.5a	4290 ± 169.4a	4042 ± 227.7a	4213 ± 270.7a
Mg (mg kg^{-1})	1799 ± 160.5a	1652 ± 143.2a	1573 ± 96.6a	1675 ± 238.9a
CEC (cmol kg^{-1})	25.7 ± 1.9a	15.57 ± 2.9b	16.47 ± 2.8b	18.7 ± 2.1b
Total Nitrogen (%)	1.0 ± 0.2a	1.1 ± 0.3a	0.9 ± 0.1a	1.0 ± 0.1a
WHC (%)	210.2 ± 23.1a	34.9 ± 5.7c	97.7 ± 12.1b	91.0 ± 10.4b
Sand (%)	43.8 ± 11.3a	52.8 ± 15.5a	46.6 ± 7.9a	46.6 ± 8.7a
Silt (%)	35.2 ± 2.7a	25.2 ± 9.5a	31.4 ± 3.5a	30.4 ± 3.9a
Clay (%)	21 ± 2.9a	22 ± 1.7a	22 ± 2.3a	23 ± 2.3a

Abbreviation: Ctrl, control; EC, electrical conductivity; OM, organic matter: CEC, cation exchange capacity; WHC, water holding capacity. Values followed by the same letter are not significantly different ($p > 0.05$).

Table 2
Plant growth attributes of two marigold varieties (TCL and AGA) grown in organic soil added with various treated materials.

Parameter		Trt Variety	Ctrl	M	B	V	BM	VM	BV	BVM
Flower number		TCL	9.0 ± 8.8a	13.2 ± 8.9a	21.3 ± 5.6a	11.6 ± 1.3a	18.8 ± 5.9a	16.0 ± 11.5a	11.6 ± 5.8a	18.3 ± 6.6a
		AGA	8.7 ± 5.9a	NA	6.3 ± 5.1a	7.8 ± 5.5a	5.3 ± 4.4a	10.3 ± 6.1a	5.4 ± 3.9a	6.8 ± 4.3a
Flower diameter		TCL	4.8 ± 0.5a	4.3 ± 0.5a	5.0 ± 0.4a	4.9 ± 0.6a	5.0 ± 0.4a	4.7 ± 0.6a	5.0 ± 0.4a	4.7 ± 0.4a
		AGA	4.9 ± 0.6a	NA	4.7 ± 0.3a	4.6 ± 0.6a	5.0 ± 0.4a	4.5 ± 0.5a	4.7 ± 0.2a	5.1 ± 1.1a
Dry biomass (g)	Root	TCL	1.7 ± 2.1ab	1.3 ± 1.0b	2.4 ± 0.9ab	2.6 ± 1.0ab	4.4 ± 2.9a	1.8 ± 1.1ab	1.5 ± 1.0ab	1.4 ± 0.7ab
		AGA	0.8 ± 0.7ab	0.1 ± 0.1b	1.2 ± 0.9ab	1.0 ± 0.5ab	1.5 ± 0.7a	1.1 ± 0.6ab	0.7 ± 0.8ab	1.2 ± 0.6ab
	Stem	TCL	12.8 ± 8.6ab	9.8 ± 7.4b	19.6 ± 6.8ab	16.6 ± 4.8ab	27.8 ± 12.3a	15.6 ± 7.3ab	13.8 ± 5.0ab	15.3 ± 10.0ab
		AGA	11.5 ± 7.3ab	1.9 ± 1.4b	11.1 ± 6.8ab	11.0 ± 5.5ab	14.8 ± 7.8ab	13.4 ± 8.3ab	11.0 ± 4.2ab	15.5 ± 5.8a
	Leaf	TCL	5.2 ± 2.7ab	3.6 ± 3.0b	7.2 ± 1.4ab	6.0 ± 1.6ab	9.8 ± 2.9a	6.1 ± 2.1ab	4.6 ± 1.4b	5.2 ± 3.1ab
		AGA	8.3 ± 4.9a	1.6 ± 1.3b	7.9 ± 3.4ab	6.6 ± 1.0ab	10.3 ± 3.1a	7.7 ± 4.0ab	7.5 ± 1.9ab	10.4 ± 3.2a
	Flower	TCL	2.5 ± 1.6a	0.9 ± 0.9a	2.5 ± 1.9a	0.8 ± 1.0a	2.3 ± 1.1a	1.5 ± 0.8a	0.7 ± 0.7a	1.4 ± 1.1a
		AGA	1.0 ± 0.7a	NA	0.3 ± 0.5a	0.5 ± 1.1a	1.4 ± 2.1a	0.7 ± 0.8a	0.5 ± 0.2a	1.4 ± 1.1a
RGR		TCL	100%	95.8%	106.9%	104.2%	111.1%	103.7%	101.3%	98.7%
		AGA	100%	76.0%	100.9%	102.1%	105.8%	103.1%	102.7%	107.4%

Trt, Treatment; TCL, Thongchalerm5022 marigold; AGA, Aga072 marigold; Ctrl, control; M, moss; B, BioVis; V, vermicompost; RGR, relative growth rate. Values followed by the same letter are not significantly different ($p > 0.05$)

Table 3
Bioactive compounds, antioxidant assays, and inhibitory effects of two marigold varieties cultivated in organic soil added with various treated materials.

Variety	Trt	Carotenoid (mg mL^{-1})	TPC (mg GAE g^{-1})	TFC (mg RU g^{-1})	ABTS (%)	DPPH (%)	FRAP (mM TE g^{-1})	α-Glucosidase (%)
TCL	Ctrl	35.3 ± 16.7 aA	91.2 ± 1.2 aAB	37.3 ± 0.5aB	96.4 ± 1.2 aA	85.1 ± 0.6 aA	33.3 ± 0.1bAB	98.3 ± 1.8 aA
	M	42.9 ± 15.5 aA	57.0 ± 0.9gI	26.3 ± 0.5eJ	60.0 ± 0.8eG	58.4 ± 1.1eI	32.9 ± 0.2cABC	86.8 ± 1.8deDE
	B	60.4 ± 12.3 aA	91.9 ± 0.4 aA	36.9 ± 0.7aB	95.1 ± 0.5 aA	83.5 ± 0.8aAB	33.4 ± 0.1bAB	86.3 ± 1.4eE
	V	54.6 ± 17.7 aA	78.4 ± 1.3eD	32.7 ± 0.6cEF	88.0 ± 1.6cC	74.0 ± 0.7cE	33.3 ± 0.1bAB	90.5 ± 1.9cC
	BM	47.9 ± 23.6 aA	88.6 ± 0.9bB	36.3 ± 0.6aB	90.4 ± 1.9bcBC	79.9 ± 1.0bCD	33.6 ± 0.1 aA	89.9 ± 1.8cdCD
	VM	47.0 ± 24.8 aA	80.4 ± 0.7dCD	34.7 ± 0.6bCD	90.1 ± 1.1bcBC	79.0 ± 0.5bD	33.2 ± 0.1bAB	98.0 ± 1.0 aA
	BV	44.7 ± 6.3 aA	71.7 ± 0.9fEF	30.9 ± 0.6dGH	82.5 ± 1.6dD	68.4 ± 1.1dF	33.2 ± 0.1bAB	94.2 ± 0.8bB
	BVM	53.8 ± 9.3 aA	82.7 ± 0.9cC	33.6 ± 1.0bcDE	91.5 ± 1.3bB	79.5 ± 1.9bD	33.2 ± 0.1bAB	96.2 ± 1.7abAB
AGA	Ctrl	30.7 ± 5.8 aA	67.9 ± 1.5dG	29.6 ± 0.7eHI	67.6 ± 1.8dF	62.1 ± 0.4eH	33.5 ± 0.0aAB	84.7 ± 1.6dE
	B	41.1 ± 17.8 aA	62.8 ± 1.2eH	28.8 ± 0.5eI	68.5 ± 1.6dF	55.8 ± 1.5fJ	32.2 ± 0.2bC	89.9 ± 1.4cCD
	V	42.6 ± 12.7 aA	74.4 ± 1.1cE	31.2 ± 0.3cdG	82.0 ± 2.3bD	64.9 ± 0.4dG	32.7 ± 1.2abBC	94.5 ± 0.8bB
	BM	38.7 ± 12.1 aA	73.1 ± 1.3cE	31.8 ± 0.3cFG	81.3 ± 1.9bcDE	69.0 ± 0.5cF	33.6 ± 0.3 aA	96.4 ± 1.2abAB
	VM	46.9 ± 6.3 aA	69.0 ± 1.0dFG	30.6 ± 0.5dGH	78.3 ± 1.5cE	65.7 ± 1.1dG	33.2 ± 0.0aAB	98.7 ± 0.6 aA
	BV	38.2 ± 12.7 aA	79.5 ± 1.1bD	34.9 ± 0.7bC	89.8 ± 1.3aBC	74.3 ± 1.1bE	33.6 ± 0.0 aA	98.7 ± 1.9 aA
	BVM	47.7 ± 9.7 aA	90.0 ± 2.8aAB	39.4 ± 0.3 aA	91.4 ± 2.0aBC	81.9 ± 1.5aBC	33.2 ± 0.1aAB	73.8 ± 1.6eF

Trt, Treatment; TCL, Thongchalerm5022 marigold; AGA, Aga072 marigold; TPC, total phenolic content; TFC, total flavonoid content; ABTS2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl hydrate; FRAP, ferric-reducing antioxidant power assay; Ctrl, control; M, moss; B, BioVis; V, vermicompost. Values followed by the same letter are not significantly different ($p > 0.05$). Lower-case letters represent differences among treatments within the same marigold variety; capital letters represent differences among all treatments of both marigold varieties.

while the lowest values (62.8 mg GAE g^{-1} and 28.8 mg RU g^{-1}) were detected in B-treated soil.

No statistically significant variation in carotenoid content was found across treatments (Table 3). Both marigold varieties had the lowest carotenoid content in the control. Mean carotenoid contents ranged from 35.3 mg mL^{-1} in the control to 60.4 mg mL^{-1} in VM-treated soil for TCL. For AGA, the lowest carotenoid content was in the control (30.7 mg mL^{-1}), whereas BM-treated soil had the highest (47.7 mg mL^{-1}).

3.4. Antioxidant activity (ABTS, DPPH and FRAP)

The TCL variety showed the maximum percentages of both ABTS (96.4%) and DPPH (85.1%) in the control, while the minimum values were in M-treated soil (60.0 and 58.4%) (Table 3). In the AGA variety, both ABTS (91.4%) and DPPH (81.9%) were highest in the same treatment, BVM-added soil, whereas the lowest ABTS (67.6%) was detected in the control, and the lowest DPPH (55.8%) in B-treated soil. Both ABTS and DPPH were higher in TCL than in AGA across all treatments. For FRAP analysis, the highest FRAP value (mM TE g^{-1}) within the TCL variety was in BM-treated soil (33.6); for the AGA variety, the highest FRAP was in VM-treated soil (33.6).

3.5. The α-glucosidase inhibitory effect

An assay for α-glucosidase inhibitory effect was characterized, and a significant variation was noted (Table 3). The percentage of α-glucosidase inhibition was highest in the control (98.3%) for TCL and in VM-treated soil (98.7%) for AGA. The lowest α-glucosidase inhibition effect for TCL was observed in B-treated soil (86.3%) and for AGA in BVM-treated soil (73.8%). The greatest inhibition potential (%) of α-glucosidase (98.3%) was observed in TCL grown under control conditions; the lowest value (73.8%) was observed in AGA under BVM treatment.

3.6. Determining Cd/Zn and phytoremediation potential

The accumulated Cd and Zn concentrations in both marigold varieties across all treatments followed the order: stem > leaf > root > flower and leaf > flower > root > stem, respectively (Fig. 1 and Table S2). No significant differences in Cd and Zn content were observed among treatments, whether for single varieties or for both varieties combined. Cadmium concentration ranged from 3.1 to 10.9 mg kg^{-1} in root, 4.8–14.4 mg kg^{-1} in stem, 3.4–9.1 mg kg^{-1} in leaf, and 2.3–3.3 mg kg^{-1} in flower. Zinc concentration ranged from 36.0 to 62.1 mg kg^{-1} in root, 34.5–62.9 mg kg^{-1} in stem, 70.4–117.6 mg kg^{-1} in leaf, and 61.1–79.1 mg kg^{-1} in flower. The highest Cd was in the AGA stem (14.4) of the B treatment, and the highest Zn was in the leaf (117.6)

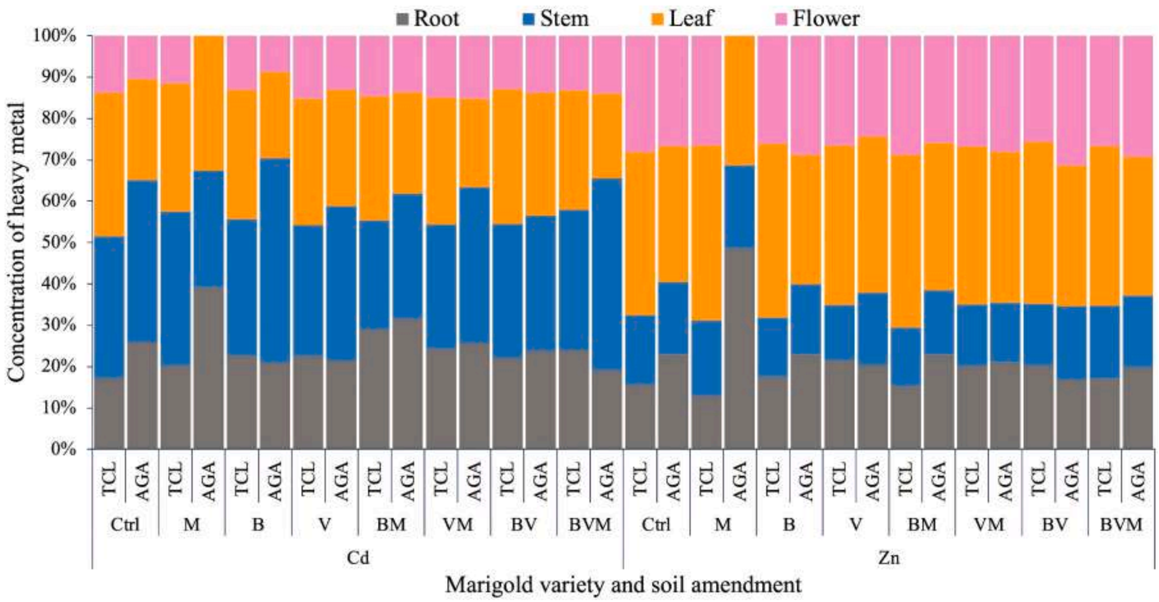

Fig. 1. Mean concentration (mg kg^{-1}) of Cd and Zn accumulated in different parts of two marigold varieties (TCL and AGA) grown in organic soil with various treated materials. Different colors represent different plant parts, and the height of each color indicates the average metal concentration. Abbreviations: *TCL, Thongchalerm5022 marigold; AGA, Aga072 marigold; Ctrl, control; M, moss; B, Biovis; V, vermicompost.*

of TCL grown in M-treated soil. Both Cd and Zn concentrations were lowest in the AGA flower (2.3 and 61.1) of BVM-treated soil. As a result of soil amendment, AGA roots and stems exhibited the highest concentrations of Cd and Zn, particularly in the control and M-treated soils. For TCL, soil and flower samples showed the highest concentrations of Cd and Zn in the control and BV-treated soils.

With the exception of BCF of Cd in TCL and Zn in AGA, both BCF and TF showed significant variation across the study (Fig. 2 and Table S3). The BCF of Cd in both TCL (1.8–4.4) and AGA (1.8–3.0) was high compared with that of Zn (0.8–1.1 and 0.8–1.1). The TF of Cd and Zn in both varieties was similar, within a range from 0.4 (Zn in M-treated soil for AGA) to 1.9 (Zn in BM-treated soil for TCL). Most TFs of Cd and Zn for both TCL and AGA varieties were > 1. Except for the BCF of Zn in AGA, most BCF values across the study were > 1.

3.7. *Comparative analysis of moss performance on HM uptake*

Cadmium and Zn uptake by moss was assessed based on the percentage of soil surface coverage achieved by the applied moss (90, 45, and 30%). Results were compared with marigold performance, including HM uptake properties by specific plant parts and soil (Fig. 3 and Table S4). Based on percentage coverage, moss Cd and Zn uptake significantly ($p < 0.05$) varied with marigold variety. Moss exhibited greater Zn uptake than Cd. Cadmium uptake by moss at all tested coverage percentages was minimal compared with that of marigold plants. In contrast, mosses had greater Zn uptake than marigolds.

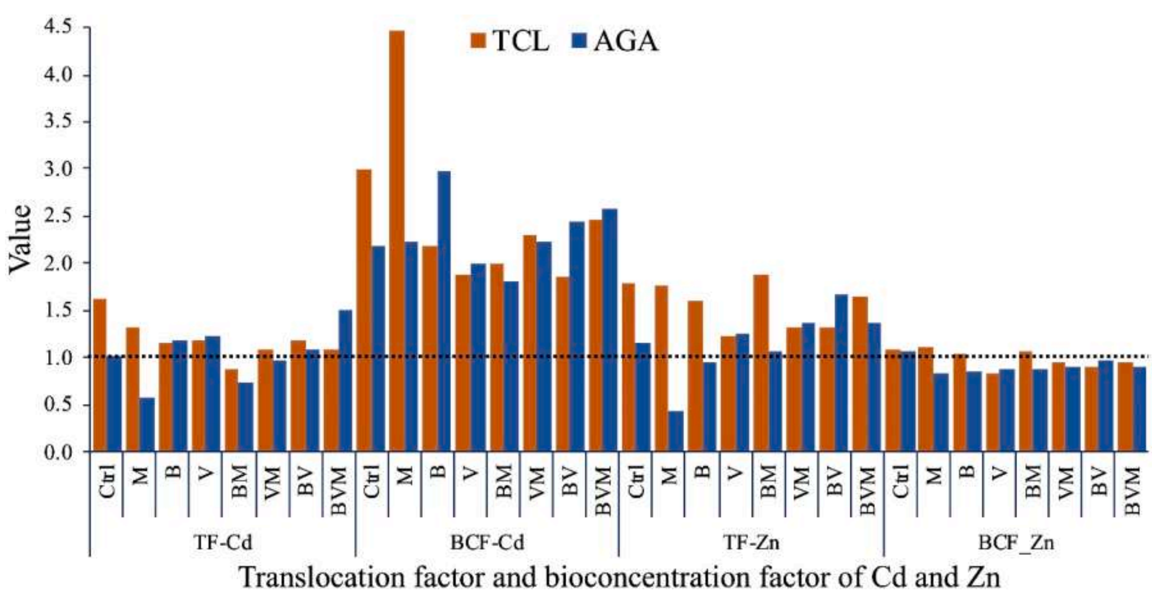

Fig. 2. Translocation factor (TF) and bioconcentration factor (BCF) of two marigold varieties (TCL and AGA), calculated from the concentrations of Cd and Zn in the samples based on different treated materials. A dotted line indicates the level at which the respective index reflects the plant's ability to transfer heavy metal (HM), such as BCF< 1 for HM excluders and TF> 1 for HM translocators. Abbreviations: *TCL, Thongchalerm5022 marigold; AGA, Aga072 marigold; Ctrl, control; M, moss; B, Biovis; V, vermicompost.*

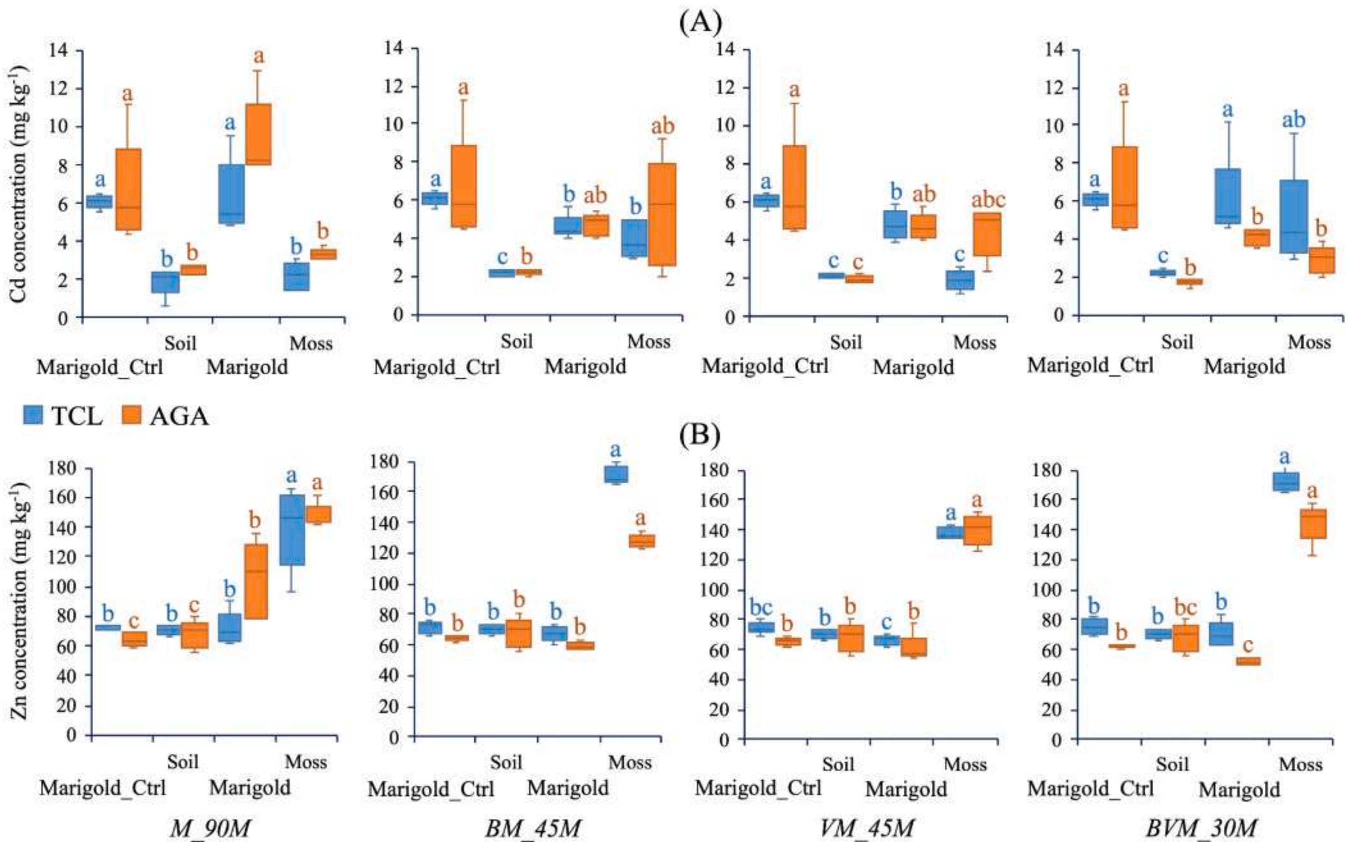

Fig. 3. Boxplots depicting Cd and Zn uptake by moss compared to marigold plants. The comparisons were performed based on percent moss coverage of the soil surface. Abbreviation: M_90M, 90% coverage of moss; BM_45M, 45% moss coverage with Biovis treatment; VM_45M, 45% moss coverage with vermicompost treatment; BVM_30M, 30% moss coverage with combined treatment of Biovis and vermicompost.

Regardless, the greatest Cd uptake (5.4 mg kg^{-1}) was observed at 45% moss coverage under the Biovis treatment. Greatest Zn uptake (147 mg kg^{-1}) was in the 90% moss coverage treatment cultivated with AGA marigold. When cultivating TCL marigold, the 30% moss coverage with combined treatment of Biovis and vermicompost resulted in the greatest uptake of both Cd (5 mg kg^{-1}) and Zn (172 mg kg^{-1}).

Compared with specific plant parts, moss at all percent coverage values exhibited the greatest Zn uptake. in all treated pots. Each respective treatment of moss had a significant ($p < 0.05$) uptake of Zn content compared with marigold plant parts, including total Zn content in the soil. The 30% moss coverage with combined treatment of Biovis and vermicompost resulted in the greatest Zn uptake (172 mg kg^{-1}) in TCL marigold; in AGA marigold, the greatest Zn uptake (147 mg kg^{-1}) was in the 90% moss coverage treatment. Cadmium uptake performance of moss was low compared with that of marigolds. Overall, moss Cd uptake was greater in AGA than in TCL marigolds across different coverage percentages. Conversely, moss performance in Zn uptake was greater in pots cultivated with TCL than in those cultivated with AGA. Nevertheless, moss added to both TCL and AGA marigolds exhibited greater Zn uptake capacity than marigolds alone (Fig. 3 and Table S4).

3.8. Relationship between plant growth attributes and amended soil properties

Mantel's test was applied to identify correlations between the physicochemical properties of the amended soils and plant responses, e.g., dry biomass and antioxidative potential (Fig. 4). In TCL, significant correlations were observed between plant parameters (biomass of stems and leaves, and flower number) and soil properties (Ava_P, Ava_K, Ca and Mg) (Fig. 4A). The only significant correlation was observed between the flower number of AGA marigold and soil OM content (Fig. 4B). Available P, Ava_K, Ca, and Mg appeared to influence plant growth in terms of flower number and dry biomass production of stems and leaves in TCL marigold. Although only a single significant correlation was observed in the AGA variety, ABTS activity and root dry biomass were marginally influenced by specific soil parameters. ABTS activity was partially correlated with soil pH, EC, Mg, and CEC, whereas root dry biomass showed weak correlations with Ava_K, Mg, and CEC.

Principal component analysis visualized the relatedness between soil properties and plant growth attributes based on treated soil amendments and moss (Figs. 4C and 4D). In TCL marigold, treatments were clustered in three separate groups, and four clusters for AGA marigold. Both PCAs showed a slight correlation between soil properties and growth attributes. Growth attributes in both marigolds, i.e., ABTS, DPPH, TFC, and TPC, were closely associated, whereas biomass and flower size (diameter) were also linked to each other, but to a lesser extent.

3.9. Correlation between dry plant biomass and plant natural biochemical

Pearson's correlation was calculated to evaluate the relationships between the dry biomass of different plant parts (root, stem, leaf, and flower) and the bioassay parameters of marigold extracts (TPC, TFC, ABTS, DPPH, FRAP, and α-glucosidase) (Table S5).

All plant bioactive compounds and antioxidant assays, except α-glucosidase, showed significant correlations ($p < 0.05$) with specific plant parameters. In the TCL variety, bioactive compounds such as TPC and TFC were significantly ($p < 0.05$) and strongly correlated with flower biomass ($r > 0.81$) and flower diameter ($r > 0.72$). The ABTS antioxidant activity in TCL showed a strong positive correlation with flower diameter, whereas DPPH activity was significantly ($p < 0.05$) correlated with flower biomass. Additionally, FRAP values in in TCL were strongly correlated with biomass of stem, leaf, and root as well as

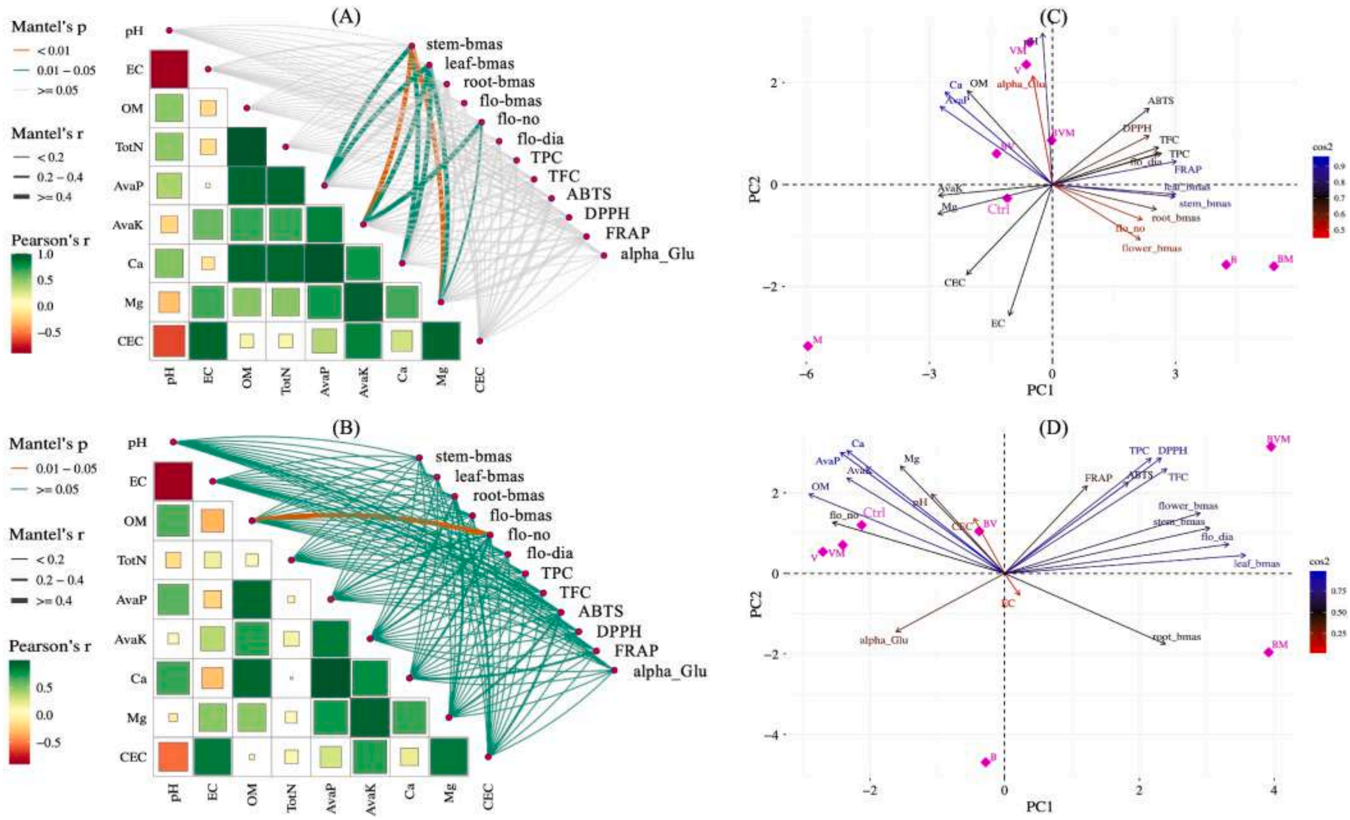

Fig. 4. Mantel's tests showing correlation matrices and principal component analyses, visualizing a clear separation of variables into clusters for different amended soil physicochemical parameters and selected plant parameters of two marigold varieties, TCL (A and C) and AGA (B and D). Abbreviations: *EC, electrical conductivity; OM, organic matter; Aval, available; CEC, cation exchange capacity; bmas, biomass; flo, flower; no, number; dia, diameter; TPC, total phenolic content; TFC, total flavonoid content; ABTS2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl hydrate; FRAP, ferric-reducing antioxidant power assay; alpha_Glu, alpha—glucosidase inhibitory activity; Ctrl, control; M, moss; B, Biovis; V, vermicompost.*

with flower diameter. In contrast, no significant correlations were observed between natural plant biochemical contents and plant growth parameters in the AGA variety.

4. Discussion

Marigold, a widely cultivated commercial flower crop, plays important multifunctional roles, serving both as primary products (whole plants, flowers) and as value-added by-products (essential oils and other bioactive extracts, including functional compounds). In addition, marigolds have been recognized for their multipurpose benefits to human health and the environment (Sharma et al., 2022). The phytoremediation potential of marigolds has gained increasing attention as a promising environmental application, particularly with regard to selecting varieties that exhibit high tolerance to contaminants and enhanced capacity for HM uptake and accumulation (Bhagwat et al., 2023; Biswal et al., 2022).

Due to the high demand for specific purposes, exploring sustainable cultural practices remains essential. Marigold cultivation with different soil amendments represents an effective integrated approach as soil fertility can be improved (Chaupoo and Kumar, 2022), and plant growth and functional performance, e.g., phytoremediation can be enhanced (Biswal et al., 2022). In the current study, physicochemical properties of the amended soils, including the non-amended control soil, varied markedly (Table 1), indicating potential impacts of applying single or combined soil amendments. The observed variations in soil properties are consistent with previous findings that different amendments effectively modulate soil physicochemical characteristics (Kaur et al., 2023).

Flower yield (number) and size (diameter), commonly referred to as yield components, are among the most used parameters to assess marigold growth performance. In the current study, TCL grown in B-treated soil and AGA grown in VM-treated soil produced the maximum number of flowers (Table 2). These findings suggest that flower number is influenced by soil fertility, although the magnitude of response may depend on the type and application rate of soil conditioners (Mann et al., 2023). Several studies have reported that different organic fertilizers played important roles in enhancing plant growth and yield attributes of marigolds (Abhishek et al., 2024; Sirai et al., 2023). In this study, with the exception of M-treated soil, both marigold varieties across all treatments showed relatively consistent RGR compared with the control. However, the TCL variety grown in BVM-treated soil had a lower growth rate than that of the control. The relatively low RGR observed in M-treated soil may be attributed to the limited short-term effects of moss application on soil fertility (Kaur et al., 2023). Although organic soil amendments often demonstrate slower short-term responses, their long term application is considered advantageous for sustainable agricultural systems, as they gradually improve soil fertility and overall soil health (Yu et al., 2024).

Marigolds are recognized as functional crops due to their phytoremediation capacity to remove HMs from contaminated areas (Swetha et al., 2023). In this study, Cd (2.3–14.4 mg kg^{-1}) and Zn (34.5–117.6 mg kg^{-1}) concentrations were measured across different marigold plant organs (root, stem, leaf, and flower). Although no statistical differences among treatments were observed within the same plant part, the stem had the highest Cd concentration (14.4 mg kg^{-1}), while the leaf had the highest Zn concentration (117.6 mg kg^{-1}). These data suggest that marigolds can accumulate Cd and Zn and may therefore be used in remediation strategies for contaminated soils (Sharma and Mathur, 2023). According to TF values, the TCL variety in all treatments, except BM-treated soil (TF < 1), revealed efficient Cd and Zn translocation (TF > 1), indicating its effectiveness in extracting HMs from contaminated soil (Biswal et al., 2022). On the other hand, the translocation efficiency of AGA was lower than that of TCL. Some treatments showed BCF < 1; however, most treatments indicated an accumulation potential (BCF > 1) of both Cd and Zn in all marigold varieties. This aligns with the findings of Madanan et al. (2021), reporting that both TCL and AGA marigolds are effective phytoextractors for Zn and Cd.

Marigolds are a rich source of antioxidants that could replace synthetic antioxidants to support human health (Chroho et al., 2021; Manivannan et al., 2021). The findings of maximum TPC and TFC in treated soils (TCL in B-treated soil and AGA in BVM-treated soil) suggest a sustainable contribution of bio-digestate (Biovis in this study) to enhance the production of bioactive compounds in marigolds (Castro et al., 2024). However, both TPC and TFC of TCL in the control showed significant increases compared with those in the other treatments. This may be attributed to the organic soil, formulated from multiple composting materials and animal manure, serving as compost and is known to enhance crop productivity, thereby promoting the biosynthesis of bioactive compounds (Ho et al., 2022). Similarly, antioxidant activities measured by ABTS, DPPH, and FRAP were highest in the control. Among the amended treatments, higher ABTS and DPPH antioxidant activities were observed in B-treated soil for the TCL variety and in BVM-treated soil for the AGA variety. These results indicate a potential association between organic amendments and antioxidant activity, highlighting the eco-friendly role of organic amendments in improving marigold cultivation, plant productivity, and bioactive compound accumulation (Salachna et al., 2021).

FRAP contents in marigolds are responsive to soil amendments (Varshney et al., 2023) and the BM treatment in this study maximized FRAP values for both marigold varieties. Inhibitory activity against α-glucosidase was markedly higher in VM-treated soil for both TCL and AGA marigolds. The α-glucosidase inhibitor is well known for its starch-blocking function for carbohydrate hydrolysis; and therefore effective in lowering blood sugar levels among people with diabetes (Parklak et al., 2023). The current finding that marigolds exhibit optimal α-glucosidase inhibitory activity suggests the potential of selected varieties for future medical studies.

Mosses provide multiple ecosystem benefits, including improved soil water retention and the sequestration of carbon and nitrogen, thereby facilitating the survival of neighboring vascular plants (Heim et al., 2014). They also influence the composition of vascular plant communities in harsh environments by modifying soil temperature, moisture content, and nutrient availability (Gornall et al., 2011). Furthermore, mosses have been reported to serve as soil amendments that modulate soil properties and enhance marigold growth (Kaur et al., 2023). In our study, moss was applied as a live plant mulch at area coverages of 90%, 45%, and 30% of the soil surface in pots, either alone or combined with other amendments. In a single moss treatment, marigold growth performance was lowest (Table 2), and values of detected secondary metabolites were also lowest among treatments (Table 3). However, Cd and Zn uptake, particularly Zn, by moss was comparable to that of the tested marigolds, including their specific plant parts (Fig. 3 and Table S4). The findings of poor plant growth attributes in single moss may be attributed to resource competition between marigolds and mosses because mosses have been shown to asymmetrically compete with vascular plants for key nutrients such as nitrogen (N) and phosphorus (P), potentially leading to reduced crop performance (Malmer et al., 2003). Another possible reason is moss-driven alterations in hydrologic processes may facilitate the encroachment of exotic species and modify soil chemistry; thereby contributing to soil degradation (Dollery et al., 2022). Conversely, mosses have demonstrated strong performance as biomonitors of potentially toxic elements in polluted environments (Baczewska-Dąbrowska et al., 2023; Urošević et al., 2023; Zinicovscaia et al., 2025). The present findings on Cd and Zn uptake by moss further confirm its high HM absorption efficiency in contaminated areas (Pradhan et al., 2017). These results also support the use of moss as a practical tool for assessing and monitoring HMs in the environment (Jiang et al., 2018; Maresca et al., 2022; Papadia et al., 2020).

Greenhouse research on marigolds grown in organic soil amended with different materials and irrigated with Cd- and Zn-contaminated water generated several valuable findings. Marigolds cultivated in non-treated (control) soil exhibited better growth performance than those grown in M-treated soil for both tested varieties. In addition, bioactive compound content, antioxidant activity, and inhibitory effects in the TCL variety were higher in the control treatment than in soils amended with single or combined materials and/or moss. These results suggest that the use of compost alone (organic soil in this study), or soil treated with single or multiple amendments, may enhance stress tolerance and improve overall plant growth (Tahiri et al., 2022). A recent report supports the current findings of improved plant growth, increased production of bioactive compounds and antioxidants, and enhanced inhibitory effects in the AGA variety grown in soil with all treated materials (BVM) (Ullah et al., 2021). Specifically, the TCL variety exhibited the highest growth performance under the B treatment, whereas the AGA variety performed best under combined B, V, and M treatments. Both TCL and AGA grown under VM treatment, which achieved maximal α-glucosidase inhibition while maintaining relatively similar growth performance (Table 2).

The secondary metabolites of marigolds are essential for both pharmacological and toxicological research. Therefore, the potential use of specific marigold varieties should be thoroughly investigated and properly managed to ensure effective use as sources of natural phytochemicals with biological activity (Burlec et al., 2021). In the current study, the TCL variety was particularly rich in TPC and exhibited strong antioxidant activity as measured by the ABTS and DPPH assays, whereas the AGA variety showed higher TFC and stronger α-glucosidase inhibitory activity. Both TCL and AGA varieties demonstrated comparable FRAP. These findings highlight the potential of different marigold varieties as valuable sources of specific phytochemicals for diverse pharmacological applications (Kar and Patra, 2022). Moreover, the higher α-glucosidase inhibitory activity observed in both varieties suggests potential applications in functional food development and medical use. Because α-glucosidase is a key enzyme involved in carbohydrate digestion, its inhibition can help reduce postprandial blood glucose levels and may contribute to the management of type 2 diabetes (Paşayeva et al., 2022).

Our findings confirm the important role of small-scale manufactured organic soil, applied individually or combined with various treated materials—including soil amendments and moss—in supporting marigold cultivation under Cd- and Zn-co-contaminated conditions. The results suggest that marigolds can accumulate varying amounts of applied Cd and Zn in specific plant organs, indicating their potential for use in phytoremediation strategies for contaminated soils. Although moss was applied as a live plant mulch, it exhibited competitive HM uptake capacity relative to marigolds. This indicates that moss not only competes for HM accumulation but may also function as an effective companion component when integrated with other soil amendments in contaminated environments, contributing to combined remediation and soil management approaches.

5. Conclusion

Marigolds are widely recognized for their multifunctional applications, including the production of bioactive compounds, phytoremediation of HMs, and ornamental values. The use of organic soil amendments plays a key role in sustainably improving soil fertility within agricultural systems. In our study, the relative growth, secondary metabolite production and bioaccumulation capacity of two marigold varieties were characterized under single- or multiple-treated materials (Biovis, vermicompost, and moss). The treated materials imparted positive effects on marigold growth and associated functional attributes.

Specifically, the TCL variety exhibited the highest levels of bioactive compounds, antioxidant activity, and enzyme-inhibitory effects in B-treated soil. In contrast, the AGA variety showed the greatest enhancement of these metabolic compounds under the combined application of BVM, indicating a variety-specific response to soil amendment strategies. In the present study, applying moss as a surface live plant mulch proved more effective when combined with other amendments than when used alone. This integrated approach enhanced marigold growth parameters by improving nutrient and water retention while simultaneously facilitating HM removal. Bioconcentration factor and TF values > 1 imply both marigold varieties possess strong phytoextraction potential of Cd and Zn. The present findings clarify the roles and benefits of applying specific soil amendments combined with moss mulch in enhancing growth performance, enriching essential phytochemicals, and promoting HM phytoaccumulation in marigolds cultivated under contaminated conditions. Together with the demonstrated efficiency of marigolds in HM uptake and translocation, these results highlight their practical potential for integrated pollution remediation strategies in contaminated environments, particularly when supported by appropriate soil amendment management practices.

CRediT authorship contribution statement

Weeradej Meeinkuirt: Writing – review & editing, Validation, Supervision, Software, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Narin Printarakul:** Methodology, Conceptualization. **Khakhanang Ratananikom:** Methodology, Investigation, Formal analysis. **Jittawan Kubola:** Methodology, Conceptualization. **Maung Thant:** Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2026.123084.

Data availability

Data will be made available on request.

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