Science of the Total Environment 919 (2024) 170791

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

# Influence of soil amendments on phytostabilization, localization and distribution of zinc and cadmium by marigold varieties

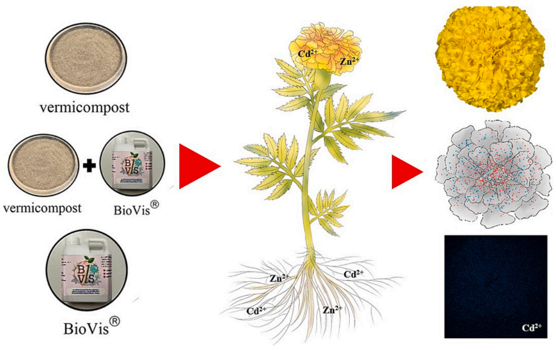
Salinthip Chunwichit[a], Theerawut Phusantisampan[b], Alapha Thongchai[c], Puntaree Taeprayoon[d], Natthapong Pechampai[e], Jittawan Kubola[f], John Pichtel[g], Weeradej Meeinkuirt[a,*]

[a] *Water and Soil Environmental Research Unit, Nakhonsawan Campus, Mahidol University, Nakhonsawan 60130, Thailand*
[b] *Department of Biotechnology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok 10800, Thailand*
[c] *Faculty of Science Technology and Agriculture, Yala Rajabhat University, Yala 95000, Thailand*
[d] *Agricultural and Environmental Utilization Research Unit, Nakhonsawan Campus, Mahidol University, Nakhonsawan 60130, Thailand*
[e] *Academic and Curriculum Division, Nakhonsawan Campus, Mahidol University, Nakhonsawan 60130, Thailand*
[f] *Department of Food Innovation and Processing, Faculty of Agricultural Technology, Buriram Rajabhat University, Buriram 31000, Thailand*
[g] *Ball State University, Environment, Geology, and Natural Resources, Muncie, IN 47306, USA*

## HIGHLIGHTS

- Biodigestate resulted in increased flower numbers.
- Vermicompost enhanced DPPH and carotenoid contents in flowers of Twenty yellow variety.
- Application of V alone increased FRAP, TFC, and TPC in flowers of the Dragon variety.
- Different marigold varieties had varying antioxidant capacities and production of bioactive compounds.
- X-ray fluorescence (XRF) spectroscopy revealed patterns of heavy metal accumulation in marigold flowers.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

Editor: Xinbin Feng

*Keywords:*
Antioxidant capacity
Bioactive compounds
Biodigestate
Heavy metals
Marigold varieties
Vermicompost

## ABSTRACT

Marigolds (*Tagetes erecta* L.) were evaluated for phytoremediation potential of cadmium (Cd) and zinc (Zn) as a function of amendment application to soil. Vermicompost (V), biodigestate (Bi), and combined V + Bi (VBi) were used as soil amendments in Zn and Cd co-contaminated soils. Application of soil amendments can alter physicochemical properties of soils, particularly pH, EC, CEC and nutrient concentrations. The VBi treatment resulted in highest percentage growth rate in biomass (52 %) for the Twenty yellow variety of marigold. Also, in the VBi treatment, leaves of Dragon yellow variety exhibited maximal accumulation of Zn and Cd. Flower extracts of Twenty yellow in the V treatment had substantial carotenoid content (71.7 mg $L^{-1}$) and lowest $IC_{50}$ value (43.7 mg $L^{-1}$), thus indicating it had highest DPPH free radical scavenging activity. Dragon yellow exhibited highest values of ferric reducing antioxidant power (FRAP; 2066 mg $L^{-1}$), total flavonoids content (TFC; 64.1 mg $L^{-1}$), and total phenolics content (TPC; 50.9 mg $L^{-1}$). Using X-ray fluorescence (XRF) spectroscopy, the atomic

---

* Corresponding author.
*E-mail address:* weeradej.mee@mahidol.ac.th (W. Meeinkuirt).

https://doi.org/10.1016/j.scitotenv.2024.170791
Received 22 November 2023; Received in revised form 19 January 2024; Accepted 6 February 2024

percentages of Zn and Cd in all marigold varieties and treatments showed similar patterns over flower surfaces, seeds, and flower petals in descending order. Prime yellow in the V treatment resulted in higher Zn accumulation in roots (bioconcentration factor of root value) > 1 and translocation factor value < 1, indicating an enhanced ability of the plant for phytostabilization. Application of V altered antioxidant activities and production of bioactive compounds as well as enhanced the excluder potential of Cd and Zn, particularly in the Prime yellow variety. Application of Bi contributed to increased flower numbers, suggesting that floriculturists cultivating marigolds for ornamental purposes may be able to generate revenue in terms of productivity and quality of flowers when marigolds are grown on contaminated land.

## 1. Introduction

Significant environmental contamination has occurred in many nations as a result of mining of ore minerals and subsequent release of heavy metals [HMs, e.g., cadmium (Cd), lead (Pb), zinc (Zn)] into water and soil (Hossen et al., 2021). Over long periods such HMs accumulate in soil; hence, they have the potential to interfere with natural soil functions and plant growth and potentially risk human health by contaminating food supplies. Heavy metals released into the environment, particularly in agricultural areas, necessitate the employment of remediation techniques to minimize and immobilize HMs in soil, while also reducing associated health risks via consumption of crops and livestock (Wuana and Okieimen, 2011).

Physical, chemical, and biological technologies have been successful in removing HMs from contaminated soil, sediment and water; however, many have limitations of high cost, excessively long time periods of operation, secondary impacts to the local environment, logistical issues, and mechanical complexity (Dhaliwal et al., 2020). Phytoremediation is a solar-driven, cost-effective in situ method of removing metals using certain green plants including forages, weeds, trees and others. This technique has been employed successfully for decades (Chowdhury et al., 2018). In recent years, ornamental flowering plants have been used for phytoremediation of HMs and other pollutants in soil and water (Ikeura et al., 2016; Woraharn et al., 2021). They have been extensively cultivated for phytoremediation purposes in both urban and rural areas, as they contribute to the creation of multi-functional landscapes as well as fulfill ecological, social, and environmental functions in contaminated terrestrial and aquatic ecosystems (Rocha et al., 2022). Marigolds (*Tagetes erecta* L.) are a good choice for cultivation on contaminated land — they have high potential to be excluders or hyperaccumulators of HMs (i.e., accumulate substantial HM concentrations in roots or shoots, respectively), and at the same time can be marketed for ornamental and other purposes (Chintakovid et al., 2008). Due to their intrinsic value, marigold flowers can aid local farmers working contaminated lands to earn greater income and provide employment for the duration of the harvest season (Chauhan et al., 2022; Kaur et al., 2022).

Agricultural soils contaminated with HMs often benefit from inputs of organic amendments (e.g., biochar, animal manures) and phosphate minerals (e.g., hydroxyapatite). These amendments lessen the potential toxicity of HMs to crops while enhancing plant tolerance and development in harsh environments (Wang et al., 2020). Biodigestate has been employed as soil amendment, either alone or with other organic amendments for crop cultivation. Application of biodigestate improves plant growth and soil physicochemical properties; this amendment is also associated with increased antioxidant capacity, total phenolics content, and ascorbic acid levels in crop plants. It can therefore aid in increasing plant resistance to HM stress (Lee et al., 2020).

Organic amendments are commonly utilized in phytoremediation strategies to facilitate and enhance the capacity of suitable plants to take up and accumulate HMs. There is a dearth of research on the efficacy of vermicompost in phytoremediation of Cd and Zn co-contaminated soils; likewise, reports on the use of biodigestate are lacking. High-producing species of marigolds were chosen for the current study in order to promote phytomanagement by emphasizing incomes (i.e., via sales from flower size and numbers or other benefits) in addition to removing HMs from contaminated soil. The primary hypothesis of the current study is that use of soil organic amendments, either separately or in combination, affect growth, uptake, and accumulation of Cd and Zn in marigold. Such investigations have not been reported elsewhere. Three commercial marigold cultivars (Dragon yellow, Twenty yellow, and Prime yellow) were assessed as potential excluder metallophytes for phytostabilization. Biodigestate (BioVis), vermicompost and biodigestate + vermicompost were evaluated as amendments to Cd- and Zn-contaminated soils. Antioxidant capacity and production of selected bioactive compounds in marigold flowers were measured. Cadmium and Zn concentrations in plant parts and soil were determined via inductively coupled plasma spectrophotometry (ICP-MS); additionally, multi-elemental imaging via X-ray fluorescence (XRF) spectrometry was employed for quantitatively measuring the elemental distribution in surfaces of whole flower tissue, and for localization of elements on surfaces of marigold petals and seeds.

## 2. Materials and methods

### 2.1. Physicochemical properties of soil samples

Soil material for this study was collected from the Mae Tao River Basin, an important agricultural region in the Mae Sot District of Tak Province. Soil was collected from locations known to be heavily contaminated with HMs (Srisawat et al., 2021). Soil samples were collected from depths of 0 to 30 cm. In the laboratory, all samples were air-dried, sieved to pass a 2-mm mesh sieve, and thoroughly mixed by hand in a plastic container. The composite soil was oven-dried at 70 °C for one week. Four soil treatments were used in this study: contaminated soil (3.15 kg) amended with vermicompost (0.35 kg or 10 % w/w) (V); contaminated soil (3.5 kg) amended with biodigestate (BioVis; 1 mL/pot/week) (Bi); contaminated soil (3.15 kg) amended with biodigestate (0.5 mL/pot/week) + vermicompost (0.35 kg) (VBi); and contaminated soil with no amendment (CT; control soil). Vermicompost was obtained from a farm in Nakhon Sawan Province, and the biodigestate was provided by Biosynthai Biotechnology Co., Ltd.

Soil pH was measured in a 1:5 soil:water (w/v) suspension using an Accumet AP115 pH meter and electrical conductivity (EC) using an EC meter (Hanna instruments; HI 993310). For each soil, particle size analysis was determined by the hydrometer method (Allen et al., 1974). Soil organic matter (SOM) content was measured using the Walkley-Black titration (Walkley and Black, 1934). Cation exchange capacity (CEC) was determined by leaching with 1 M ammonium acetate ($NH_4C_2H_3O_2$) at pH 7 (Ross et al., 2008). Total N was measured using the Kjeldahl method (Black, 1965); available P was analyzed by the Bray II method (Bray and Kurtz, 1945), followed by analysis on an inductively coupled plasma optical emission spectrophotometer (ICP-OES, Varian 720-ES). Available K was determined by ICP-OES after 1 M $NH_4OAC$ extraction. 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 contents using ICP-OES (APHA, AWWA, WEF, 2005). One-half gram of soil sample was microwave digested (ETHOS One; Milestone Inc.) with concentrated 70 % $HNO_3$ and 30 % $H_2O_2$, after which total soil Cd and Zn contents were determined using ICP-OES. NIST 1515 apple leaves and 2711a Montana soil were used for quality control in plant and soil analysis (90–110 % recovery, respectively).

### 2.2. Greenhouse study

Seeds of three cultivars of marigold, including Dragon yellow, Twenty yellow, and Prime yellow, were purchased from the AGA Agro Co. Ltd., Chiang Mai, Thailand. A uniform size of marigold seeds was selected and sterilized for 5 min in 10 % sodium hypochlorite (NaOCl), then thoroughly rinsed twice in deionized water (DI). Seeds were allowed to germinate for one week in peat moss having HM levels below detectable levels. When seedlings reached a height of approximately 5.2 ± 0.6 cm they were transplanted into pots in the greenhouse of Mahidol University, Nakhonsawan campus. Temperatures in the greenhouse ranged from 27 to 32 °C, relative humidity from 65 to 85 %, and light intensities from 5000 to 16,000 lx, respectively.

Each soil treatment was placed into 3.5-L plastic pots in five replicates. All pots were incubated at 25 °C in complete darkness for 16 days in a climatic chamber (F.lli Della Marca s.r.l, Italy) before moving to the greenhouse. At the start of the experiments, DI water was applied to attain 70 % water holding capacity (WHC) (Alaboudi et al., 2018). A randomized complete block design (RCBD) was used to arrange pots on the bench before planting marigolds. A single healthy seedling was chosen and cultivated until harvest at approximately three months. All pots were supplemented every 15 days with 3 g of Osmocote™ controlled-release fertilizer (16-16-16) to maintain adequate levels of essential nutrients. Marigolds were irrigated twice daily with a 108.9 mL of solution containing 0.064 mg Cd $L^{-1}$ and 0.047 mg Zn $L^{-1}$. Cadmium and Zn concentrations in the solution were similar to those determined in the Mae Tao stream located near contaminated soils in the Mae Tao River Basin.

### 2.3. Cadmium and Zn determination

Following plant harvest, marigolds were thoroughly rinsed multiple times with tap water to remove adhering soil and then rinsed two or three times with DI water. Plant material was separated into roots, stems, leaves and flowers. Tissue was oven-dried for five days at 70 °C, ground to a fine powder using a mortar and pestle (IKA; A11 basic), sieved through a 1-mm mesh sieve, and weighed. One-half gram of each dried plant component was placed in a vessel tube with conc. 37 % hydrochloric acid (HCl) and conc. 70 % nitric acid ($HNO_3$) for microwave digestion (ETHOS One; Milestone Inc.). The digested solution was analyzed for Cd and Zn using ICP-MS (NexlON 2000; Perkin Elmer, USA) (APHA, AWWA, WEF, 2005). A soil sample was collected from each pot in order to determine levels of Cd and Zn. Soil material was oven-dried at 80 °C for 7 days, ground with a mortar and pestle and then sieved through a 1-mm mesh sieve. One-half gram of dried soil was digested with conc. 70 % $HNO_3$ and 30 % hydrogen peroxide ($H_2O_2$) using microwave digestion. Cadmium and Zn concentrations were determined by ICP-MS. To ensure accuracy of data, NIST 1515 apple leaves, 2711a Montana soil, and reagent blanks were used to calculate percentage recovery for plant and soil samples. Percentage recovery of Cd and Zn ranged from 91.5 ± 4.5 % to 106.4 ± 21.2 % and 94.3 ± 6.7 % to 108.5 ± 11.3 %, respectively.

### 2.4. Elemental mapping and localization in marigold flower tissues

X-ray fluorescence (XRF) was employed for microchemical mapping and element distribution on the entire surface of flowers (3–8 cm diameter), while X-ray spectra of flower petal surfaces, and seeds on five targeted points (or 5 replicates) were also determined. Plant tissue was oven-dried for one week at 40 °C before being placed on a type-affixed acrylic and analyzed using the X-ray fluorescence analytical microscope (XRF; HORIBA Scientific, Indonesia). Distribution and localization of K, Ca, P, Fe, Zn, and Cd were analyzed in marigold flowers, and surfaces of flower petals and seeds. The X-ray tube was operated at 50 kV, 3932 s and 1000 μA and 30–50 kV, 100 s and 1000 μA for the plant flower surface, and each specified surface of plant flowers (petal and seed), respectively (Taeprayoon et al., 2023).

### 2.5. Analysis of bioactive compounds and antioxidant activity in marigold flowers

#### 2.5.1. Bioactive compounds

Total phenolics content (TPC) in marigold flowers was determined by the Folin-Ciocalteu colorimetric method (Tinrat, 2016) with some modification, using gallic acid as a standard. Five grams of dried marigold flowers were immersed in 95 % ethanol for 7 d before being concentrated in a rotary evaporator. For marigold flower extract, 0.01 g of crude extract was dissolved in 1 mL of distilled water. A 96-well microplate (ThermoFisher Scientific, Life Technologies Holdings Pte Ltd., Singapore) was filled with the mixture, which also included 50 μL of fresh Folin-Ciocalteu reagent and 50 μL of marigold flower extract. A total of 100 μL of 0.175 M sodium hydroxide (NaOH) was added to the mixture and left in the dark for 3 min. Absorbance at 754 nm was measured using a microplate reader. TPC content in each extract was expressed as mg gallic acid equivalents in 1 g of dried sample, using the linear equation based on the standard calibration curve of gallic acid (0.05–0.5 mg $mL^{-1}$). Each determination was carried out in five replicates.

Total flavonoids content (TFC) in marigold flowers was analyzed by the aluminum chloride ($AlCl_3$) colorimetric method (Tinrat, 2016) with some modification, using rutin as a standard. The mixture contained 50 μL of flower extract which was mixed with 350 μL of DI water. Thirty microliters of 7.5 % w/v sodium nitrite ($NaNO_2$) were added to the mixture, which was placed in the dark for 5 min. After thoroughly mixing in 300 μL of 1 % w/v $AlCl_3$ and keeping the mixture in the dark for 6 min, 200 μL of 4 % w/v NaOH was added. The solution was thoroughly mixed with DI water and brought to 1000 μL, then kept in the dark for approximately 15 min. Absorbance at 510 nm was measured using the microplate reader. The presence of flavonoids was indicated by a yellow color. The TPC value in each extract was expressed as mg rutin equivalents in 1 g of dried sample, using a linear equation based on a calibration curve of rutin (0.05–0.5 mg $mL^{-1}$). Each determination was carried out in five replicates.

Extraction of carotenoids was carried out following Lichtenthaler (1987) with some modification. The dried marigold flower (0.2 g) was extracted with 10 mL of 80 % acetone, then the solution filtered with Whatman No. 42 filter paper. Three milliliters of 80 % acetone were added and the solution was centrifuged at 7825 ×*g* for 5 min. Three milliliters of the supernatant were transferred to a cuvette and absorbance measured at 663 nm (chlorophyll *a*), 646 nm (chlorophyll *b*) and 470 nm (carotenoids) using a UV–visible spectrophotometer. Pigment concentrations were calculated as follows:

$$\text{Chlorophyll } a \left(\text{mg mL}^{-1}\right) = (12.5 \times \text{A}_{663}) - (2.79 \times \text{A}_{646})$$

$$\text{Chlorophyll } b \left(\text{mg mL}^{-1}\right) = (21.51 \times \text{A}_{646}) - (5.1 \times \text{A}_{663})$$

$$\text{Chlorophyll } T \left(\text{mg mL}^{-1}\right) = \text{Chlorophyll } a + \text{Chlorophyll } b$$

$$\text{Carotenoids} \left(\text{mg mL}^{-1}\right) = (1000 \times \text{A}_{470}) - (1.8 \times \text{Chlorophyll } a) - (85.02 \times \text{Chlorophyll } b)$$

#### 2.5.2. Antioxidant activity

The FRAP (ferric reducing antioxidant power) assay was performed as described by Benzie and Strain (1996) with some modification. The FRAP reagent was initially prepared with 300 mM acetate buffer (pH 3.6), 10 mM 2,4, 6-tripyridyl-*s*-triazine (TPTZ) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution in a ratio of 10:1:1 (v/v/v). An aliquot of 50 μL marigold flower extract was mixed with 150 μL of the FRAP reagent in a 96-well microplate. Absorbance was measured at 596 nm using the microplate reader after incubation in the dark for 30 min. Results were expressed as ascorbic acid equivalents in milligram per gram dry weight (mg AAE $g^{-1}$ DW) based on a calibration curve of ascorbic acid (0.1–10 mg $mL^{-1}$). Each determination was carried out in five replicates.

For the quantitative measurement of radical scavenging properties, the 96-well microplate assay was used. Free radical scavenging ability of flower extracts was tested by the DPPH radical scavenging assay as described by Fenglin et al. (2004). A stock solution (1 mg $mL^{-1}$) of each extract was prepared via dilution with ethanol at different concentrations. After preparing a fresh working solution of DPPH in ethanol, an aliquot of 100 μL was added to each well following incubation for 30 min in the dark. The microplate reader was used to measure quenching at an absorbance of 517 nm. Each determination was carried out in five replicates. Furthermore, $IC_{50}$ values were used to express the free radical-scavenging activities of test samples and the positive control (ascorbic acid). DPPH scavenging activity was calculated using the following formula.

$$\text{DPPH radical scavenging activity }(\%) = \frac{(\text{absorbance of control} - \text{absorbance of sample})}{\text{absorbance of control}} \times 100$$

### 2.6. Data analysis

Plant growth performance attributes including dry biomass of each plant part, percentage growth rate in height, root collar diameter (RCD) and dry biomass, as well as flower diameter and flower numbers, were measured (Köksal et al., 2017; Thongchai et al., 2021).

$$\text{Percentage growth rate }(\%) = \frac{\text{Selected growth data after harvest} - \text{Selected growth data before harvest}}{\text{Total days}} \times 100$$

The metal translocation factor (TF) index describes how HMs are transferred from soil via the root system and into above-ground parts. This factor can indicate the potential for phytoremediation of the tested plants, as a TF value $> 1$ indicates that plants may be hyperaccumulators. The bioconcentration factor (BCF) shows the potential of HM accumulation in the specific plant part.

$$\text{TF} = \frac{\text{HM concentration in shoot (mg/kg)}}{\text{HM concentration in root (mg/kg)}}$$

$$\text{BCF} = \frac{\text{HM concentration in shoot or root (mg/kg)}}{\text{Extractable HM concentration in soil (mg/kg)}}$$

Statistical comparison between sample groups was performed using one-way ANOVA followed by Least Significant Difference ($p < 0.05$). All data was expressed as mean ± SD.

## 3. Results and discussion

### 3.1. Physicochemical properties of soils

Textures of the V and Bi soils were sandy clay loam and clay loam, respectively, whereas VBi and CT soils were loam (Table 1). Loam is a typical soil texture at the sampling location and is often ideal for productive crop growth (Saengwilai et al., 2020). Soil pH was slightly alkaline (pH 7.3–7.6) in both amended and control soils. Certain plant types grow satisfactorily in neutral to alkaline soil (pH 7.0–7.3), as determined for the V and Bi treatments; in many plants, however, growth is hindered in alkaline soil (Gentili et al., 2018). Soil with neutral to alkaline pH (e.g., VBi) will not allow substantial leaching of Cd and Zn and tend to decrease HM bioavailability (Lu et al., 2020). Soil EC values ranged from 0.4 to 0.7 dS $m^{-1}$. The highest EC value (V treatment) is attributed to enhanced solubility and mobility of minerals as well as decomposition of OM and mineralization of compounds (Amouei et al., 2017). Electrical conductivity in the soils were low, and thus ideal for plant growth. Once the amendment and soil were mixed, salinity was further diluted.

Organic matter (OM) content is crucial for supporting plant growth and soil health by enhancing soil physicochemical and biolological functions. The V and Bi treatments had high OM content, suggesting that vermicompost or biodigestate supplements may improve physicochemical properties of the affected soil. Vermicomposting uses African night crawler (*Eudrilus eugeniae* (Kinberg)) to decompose organic wastes like Chinese cabbage, kale, lettuce, and dairy cattle manure. The biodigestate was produced by anaerobic digestion of organic waste (i.e., food scraps) and was rich in nutrients. The application of soil amendments increased CEC values modestly in V and Bi soils (9.9 and 10.5 cmol $kg^{-1}$, respectively); CEC levels in VBi and CT soils were lower (8.5 and 8.7 cmol $kg^{-1}$, respectively). The substantial CEC, a result of amendment additions, can greatly enhance binding to metallic cations including nutrients (Olorunfemi et al., 2016; Yu et al., 2023). Increased CEC in the V and Bi soils is likely linked to elevated OM content. A high CEC can imply that the soil has a high nutrient content—particularly total N and avail. P, as well as a good soil structure and increased microbial activity, all of which contribute to robust soil ecology. A good capacity to retain nutrients is determined in CEC $> 10$ cmol $kg^{-1}$ (for example, as demonstrated in Bi treatment), which is suitable for plant growth (Chowdhury et al., 2021). Furthermore, CEC is can be employed for predicting soil productivity and for recommending actions that can reduce the need for costly soil amendments and interventions (Ćirić et al., 2023). Additions of organic amendments improve soil water holding capacity (WHC) (Herawati et al., 2020); this was revealed by a slight increase in WHC in soil amended with the vermicompost and biodigestate (Table 1). The capacity of amendments to increase organic content and WHC ultimately aids in the immobilization of HMs in the soil (Hegade et al., 2023). The recommended soil WHC content for optimal agricultural production is approximately 50 %; this level is appropriate for optimizing both C and N mineralization (Franzluebbers, 2020). The amended soils in this study did not raise soil WHC content to desired levels, however — values ranged from 34.5 to 37 %.

The vermicompost treatment increased levels of total N, available P and ext. Mg by 1.3×, 2.9× and 1.6×, while biodigestate likewise increased total N, avail. P and ext. Mg levels. The ext. Ca concentration in the contaminated soil increased only marginally, however, using

**Table 1**
Physicochemical properties of soils ($n = 5$).

| Parameter | Unit | Soil | | | |
|---|---|---|---|---|---|
| | | V | Bi | VBi | CT |
| Texture | | Sandy clay loam | Clay loam | Loam | Loam |
| Sand | % | 47.0 | 44.0 | 45.0 | 45.0 |
| Silt | % | 27.6 | 28.6 | 28.8 | 29.6 |
| Clay | % | 25.4 | 27.4 | 26.2 | 25.4 |
| pH | | 7.3 | 7.3 | 7.6 | 7.5 |
| EC | dS m$^{-1}$ | 0.7 | 0.5 | 0.3 | 0.4 |
| OM | % | 3.0 | 3.0 | 2.4 | 2.4 |
| CEC | cmol kg$^{-1}$ | 9.9 | 10.5 | 8.5 | 8.7 |
| WHC | % | 37.0 | 38.3 | 34.5 | 35.4 |
| Total N | % | 0.15 | 0.15 | 0.12 | 0.12 |
| Avail. P | mg kg$^{-1}$ | 209.0 | 132.0 | 48.0 | 73.0 |
| Avail. K | mg kg$^{-1}$ | 185.0 | 142.0 | 106.0 | 168.0 |
| Ext. Ca | mg kg$^{-1}$ | 4166.0 | 4127.0 | 4269.0 | 4053.0 |
| Ext. Mg | mg kg$^{-1}$ | 375.0 | 326.0 | 231.0 | 231.0 |
| Total Cd | mg kg$^{-1}$ | 22.0 ± 2.1 | 14.4 ± 1.4 | 21.9 ± 1.4 | 25.0 ± 3.2 |
| Total Zn | mg kg$^{-1}$ | 1003.6 ± 63.6 | 676.3 ± 66.4 | 1018.5 ± 79.7 | 1109.0 ± 107.4 |
| Ext. Cd | mg kg$^{-1}$ | 19.5 ± 1.7 | 13.4 ± 1.4 | 15.6 ± 0.5 | 15.6 ± 0.4 |
| Ext. Zn | mg kg$^{-1}$ | 938.9 ± 50.2 | 656.9 ± 46.1 | 773.0 ± 18.6 | 810.5 ± 33.9 |

V = soil + vermicompost, Bi = soil + biodigestate, VBi = soil + vermicompost + biodigestate, CT = contaminated soil without amendment (control soil), 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.

combined vermicompost and biodigestate. The VBi treatment had the lowest P content (48 mg kg$^{-1}$); however, a concentration of available P in loam soil >26.3 mg kg$^{-1}$ is considered sufficient for crop growth (Haque et al., 2013). The biodigestate treatment furthermore resulted in reduced total Zn and Cd concentrations by approximately 1.6 and 1.7×, respectively. In this study, vermicompost and biodigestate were considered suitable soil amendments which promote growth and development of agricultural crops. Concentrations of extractable Zn and Cd, however, were reduced only slightly with amendment application. Zinc concentrations in all soil treatments were within the threshold and guideline limits for metals in soils (200 mg kg$^{-1}$), whereas Cd concentrations were in excess of levels specified by MEF (2007).

### 3.2. *Growth performance of marigolds*

Percentage growth rates in dry biomass of the marigold varieties were in the order: Twenty yellow > Prime yellow > Dragon yellow (Table 2). Trends in the dry biomass of stems and flowers are likewise supported by this data. When compared to data for soil treatments among other marigold varieties, Twenty yellow had highest dry biomass of stems and highest growth rate in dry biomass values, particularly in the VBi treatment ($p < 0.05$), indicating that dry biomass values of marigold shoots were more significant in reflecting growth rate in dry biomass. However, only the combined vermicompost and biodigestate treatment for the Twenty yellow variety demonstrated appreciable growth performance. Although soil in the VBi treatment exhibited lower values of desirable growth parameters, it had the highest concentrations of ext. Ca and high concentrations of avail. P. The lowest EC and Cd values reveal the benefits of the combined vermicompost and biodigestate as a diluent, which may improve plant adaptation in contaminated soil, ultimately increasing plant growth.

In contrast to other marigold varieties, leaves of the Twenty yellow variety showed significantly high dry biomass ($p < 0.05$), particularly in the Bi treatment, and values for leaves of Prime yellow and Dragon yellow were lower (7–11.7 g). Although growth performance of Twenty yellow was notable, dry biomass of roots and growth rate in root collar diameter (RCD) were somewhat low following application of amendments to soil. Marigold height may not be an accurate reflection of growth performance. In comparison to other marigold varieties, Prime yellow from all soil treatments had a slightly higher growth rate in height. The Bi treatment experienced highest growth rate in height ($p < 0.05$). Twenty yellow and Dragon yellow had comparable heights (46.7–55.2 %). The greater marigold height may have been a result of marigold variety and fertilizer or soil amendment application.

In recent years, biodigestate products have been utilized to increase crop productivity and soil fertility (Lee et al., 2020); however, few accounts of its usage in Thailand are documented, despite the fact that this nation has an extensive agricultural base and ample resources to produce biogas along with the nutrient-rich digestate as a by-product. The current study is the first in Thailand to apply biodigestate for a phytoremediation approach. According to the data provided herein, amending contaminated soil with biodigestate is beneficial. Growth rate in dry biomass for all varieties was enhanced by 1.1–1.3 times when compared to control treatments; these results are indicative of its potential to support plant growth performance. The vermicompost treatment, on the other hand, resulted in lower plant growth performance with the exception of Twenty yellow, whose growth rate in dry biomass value was marginally greater than in the Bi treatment (50.9 %). BioVis, a newly developed product, improves soil physicochemical properties and stimulates root germination and production of plant hormones (Enzmart Biotech, 2024). According to the current study, robust root extension may effectively absorb greater quantities of nutrients and lead to enhanced growth performance.

Marigold varieties did not differ markedly in flower diameter (5.3–7.3 cm); however, Dragon yellow had slightly greater flower diameter across all treatments (5.8–7.3 cm), with the greatest value in the Bi and VBi treatments ($p < 0.05$). Flower diameters of all marigold varieties were comparable to that for the control treatment ($p > 0.05$); it is thus likely that the amendments used in this study did not encourage increased flower diameter. The variations in flower diameter among marigold species may be caused by plant genotype, which in this study were the main effects (Tork et al., 2022), rather than soil amendments or HM concentration. Flower size decreased significantly, however, in the V treatment for Prime yellow and Dragon yellow ($p < 0.05$). This implies that this vermicompost treatment was ineffective in increasing flower size while also failing to mitigate the impacts of the HMs. Heavy metals diminish photosynthetic activity by inducing oxidative stress; these results are in accordance with the results of Aravindhan et al. (2019).

Dragon yellow in the Bi treatment produced the greatest number of marigold flowers (22.2; $p < 0.05$) compared to other marigold varieties; however, the similar flower number in the control treatment ($p > 0.05$) implies that soil amendment had no effect on increasing number of flowers. The Twenty yellow variety in all soil amendments had increased number of flowers. The Bi treatment ($p < 0.05$) had highest flower numbers; however, the result was only marginally different from other amended treatments ($p > 0.05$). The tested plants produced significantly more flower numbers than did marigolds from previous reports (American, French, Babuda, Honey, and Sunshine), and the flower size of Dragon yellow was comparable to that previously reported for the Sunshine variety under the same greenhouse conditions (Thongchai et al., 2019). This study indicates that soil P concentration and presence of plant hormones (e.g., salicylic acid; unpublished data) in the biodigestate are the primary factors influencing flower growth and numbers. Clay loam texture typically exhibits better aggregation and experiences lower soil drainage and porosity compared to loam and sandy loam (Paul and Lee, 1976). Furthermore, good water management

**Table 2**
Growth performance of the tested marigold varieties ($n = 5$).

| Treatment | Dry biomass | | | | Percentage growth rate (%) | | | Flower diameter (cm) | Flower number |
|---|---|---|---|---|---|---|---|---|---|
| | Stem (g) | Leave (g) | Root (g) | Flower (g) | Height | Root collar diameter | Dry biomass | | |
| PV | 10.9 ± 2.7aEF | 7.0 ± 1.6aD | 5.3 ± 0.9bcBCD | 8.2 ± 1.4bC | 68 ± 4.9bB | 0.7 ± 0.1aAB | 34.9 ± 2bcEF | 5.3 ± 0.4bE | 18.0 ± 3.2bC |
| PBi | 12.8 ± 2.6aCDE | 7.5 ± 1.8aD | 9.3 ± 1.5aA | 11.8 ± 1.3aAB | 74.2 ± 4.2aA | 0.7 ± 0.1aE | 45.9 ± 3.7aBC | 5.5 ± 0.2abCDE | 22.2 ± 2.5aA |
| PVBi | 10.8 ± 0.9aEF | 7.5 ± 2.1aD | 4.0 ± 0.8cDE | 6.4 ± 0.5cD | 65.7 ± 5.7bB | 0.7 ± 0.1aAB | 31.8 ± 1.2cFG | 5.7 ± 0.3aCD | 18.0 ± 1.4bC |
| PCT | 11.7 ± 1aDE | 7.7 ± 1.5aD | 6.8 ± 1.7bBC | 8.5 ± 0.6bC | 70.1 ± 1.9abAB | 0.7 ± 0aABC | 38.4 ± 3.2bDE | 5.5 ± 0.3abCDE | 21.4 ± 1.5aAB |
| TV | 16.4 ± 5.1abB | 12.1 ± 1.9aAB | 4.6 ± 1.5aDE | 12.8 ± 1.4aA | 46.7 ± 6bE | 0.4 ± 0.1bE | 50.9 ± 8.8abAB | 6.5 ± 0.4aB | 18.8 ± 2.0aC |
| TBi | 15.2 ± 3.3bBC | 14.3 ± 2.8aA | 3.9 ± 1.4aDE | 11.1 ± 0.7bB | 55.2 ± 2.2aC | 0.5 ± 0aE | 49.3 ± 5.4abAB | 5.9 ± 0.5bC | 19.4 ± 1.1aBC |
| TVBi | 21.2 ± 2.8aA | 11.7 ± 1.5aB | 3.1 ± 1.7aE | 10.8 ± 0.5bB | 53.4 ± 5.7aCD | 0.5 ± 0.1aF | 52.0 ± 3.6aA | 5.4 ± 0.3bDE | 18.6 ± 0.9aC |
| TCT | 14.3 ± 2.7bBCD | 12 ± 2.5aAB | 4.1 ± 1.2aDE | 8.7 ± 0.8cC | 55.2 ± 3.2aC | 0.6 ± 0.1aCDE | 43.3 ± 4.9bCD | 5.8 ± 0.3bCD | 12.2 ± 1.1bD |
| DV | 10.7 ± 1.1aEF | 11.7 ± 3aAB | 5.7 ± 2aBCD | 6.2 ± 0.4aD | 47 ± 1.7cE | 0.6 ± 0aCDE | 38.1 ± 4.5aDE | 5.8 ± 0.3bCD | 18.0 ± 1.2aC |
| DBi | 10.7 ± 1.6aEF | 10.5 ± 1.4abBC | 7.0 ± 1.7aB | 6.6 ± 0.3aD | 54.7 ± 4aCD | 0.6 ± 0.1aDE | 38.6 ± 2.7aDE | 7.3 ± 0.5aA | 9.2 ± 0.4cE |
| DVBi | 6.7 ± 1bG | 8.2 ± 1.8bCD | 5.1 ± 1.4aCD | 6.7 ± 0.8aD | 49.7 ± 2.6bcDE | 0.6 ± 0.1aBCD | 29.6 ± 4.5bF | 7.3 ± 0.2aA | 11.2 ± 1.3bDE |
| DCT | 8.2 ± 1.8bFG | 8.9 ± 1.7bCD | 2.8 ± 1.1bE | 6.3 ± 0.5aD | 50.9 ± 2.4bCDE | 0.6 ± 0.1aDE | 29.0 ± 2.5bG | 7.2 ± 0.2aA | 10.8 ± 0.8bDE |

P = Prime yellow, T = Twenty yellow, D = Dragon yellow, V = soil + vermicompost, Bi = soil + biodigestate, VBi = soil + vermicompost + biodigestate, CT = contaminated soil without amendment (control soil) 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, whereas capital letters represent differences among growth performance of all plant species and all treatments.

in pot systems, including avoiding excessive moisture, is a key element that for enhancing marigold growth and production (Younis et al., 2018).

Determination of flower diameter and number of flowers is one of the main goals of the current study. Flower production in contaminated soil may serve as a source of income for local villagers; such crops could replace current edible crops that are documented to be contaminated with HMs. Despite the fact that Zn mining ceased as of 2016, numerous villagers in the Mae Tao River Basin have been afflicted with Cd-related diseases as a result of long-term consumption of local crops (Taeprayoon

**Table 3**
Zinc and Cd accumulation ($\text{mg kg}^{-1}$) in plant parts ($n = 5$).

| Treatment | Flower | | Stem | | Root | | Leaf | | Whole plant | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Zn | Cd | Zn | Cd | Zn | Cd | Zn | Cd | Zn | Cd |
| PV | 25.1 ± 1.4aG | 2.7 ± 0.2aA | 23.4 ± 3.4cDE | 14.4 ± 2.3aCD | 121.3 ± 11.3aA | 65.7 ± 9.4aA | 142.5 ± 36bCD | 13.2 ± 3.3aCD | 43.8 ± 3.3cD | 22.2 ± 11.2aA |
| PBi | 24.7 ± 1.2bcG | 1.9 ± 0.2cCD | 28.4 ± 2.9bC | 16.8 ± 3.6aABCD | 89.9 ± 4.8bcCD | 37.0 ± 2.9bcC | 126.0 ± 16bcDE | 9.1 ± 3.3bD | 60.3 ± 5.3aABC | 16.5 ± 1.9abBC |
| PVBi | 26.5 ± 1.1cFG | 2.2 ± 0.4bcBC | 44.0 ± 2.3aB | 15.8 ± 2.1aBCD | 83.0 ± 5.7cDEF | 31.5 ± 2.2cD | 104.9 ± 26cE | 7.5 ± 0.9bD | 53.5 ± 5.3abCD | 13.1 ± 3.7bBCD |
| PCT | 27.7 ± 1.8bF | 2.5 ± 0.3abAB | 22.4 ± 1.7cE | 13.7 ± 1.2aCDE | 96.1 ± 9.2bC | 40.4 ± 2.8bBC | 179.9 ± 15.4aAB | 9.9 ± 0.7bD | 51.0 ± 11.1bcCD | 13.7 ± 3.7bBCD |
| TV | 35.5 ± 1.4aAB | 2.7 ± 0.3aA | 22.7 ± 1.4bE | 19.5 ± 1.3aA | 89.5 ± 3.4aCDE | 44.9 ± 0.5aB | 165.1 ± 52.8aBC | 21.0 ± 5.8aCD | 53.2 ± 5.1aCD | 17.8 ± 4.4aAB |
| TBi | 33.2 ± 1.1aBC | 1.9 ± 0.1bCD | 21.9 ± 1.8bE | 13.4 ± 2.3bDE | 68.9 ± 3.1cG | 25.6 ± 0.4cE | 104.4 ± 21.2bE | 13.1 ± 9.9abCD | 49.7 ± 3.1aCD | 11.4 ± 1.1bCD |
| TVBi | 34.1 ± 3.7aAB | 2.0 ± 0.4bCD | 49.7 ± 3.4aA | 18.9 ± 3.9aAB | 70.0 ± 6.5cG | 24.4 ± 0.2cE | 119.6 ± 31.6abDE | 11.7 ± 1.2bCD | 45.3 ± 3.2aD | 11.3 ± 0.9bCD |
| TCT | 36.6 ± 3.2aA | 2.7 ± 0.1aA | 20 ± 2bE | 14.1 ± 1.7bCDE | 79.8 ± 11.5bEF | 30.4 ± 5.6bD | 146.4 ± 36.7abBCD | 13.8 ± 3.7abBCD | 55.2 ± 14aBCD | 14.1 ± 1.9bBCD |
| DV | 30.6 ± 2.5abDE | 2.6 ± 0.5aA | 27.1 ± 6.1aCD | 17.2 ± 4.3aABC | 106.6 ± 6.5aB | 43.2 ± 2.6aB | 171.7 ± 19.3bBC | 18.9 ± 3.6abABC | 55.5 ± 9.3abBCD | 16.1 ± 3.8abBCD |
| DBi | 33.0 ± 2.0aBCD | 2.0 ± 0.2cCD | 19.9 ± 0.8bE | 17.1 ± 3aABC | 73.7 ± 10.3bFG | 23.8 ± 3.7cE | 110.6 ± 23.7cDE | 17.7 ± 11.8abABC | 71.8 ± 27.9aA | 12.9 ± 2.7bcBCD |
| DVBi | 30.8 ± 1.5abCDE | 2.0 ± 0.2bCD | 28.7 ± 4.8aC | 17.1 ± 3aABC | 97.3 ± 7.5aBC | 31.1 ± 1.5bD | 208.7 ± 32.3aA | 24.1 ± 10.8aA | 68.4 ± 15.8abAB | 18.0 ± 2.2aAB |
| DCT | 30.4 ± 1.6bE | 1.7 ± 0.1cD | 22.4 ± 5.4abE | 10.9 ± 2.6bE | 74.4 ± 6.5bFG | 22.3 ± 0.3cE | 92.1 ± 3.5cE | 9.3 ± 1.6bD | 48.3 ± 9.2bCD | 10.7 ± 3.3cD |

P = Prime yellow, T = Twenty yellow, D = Dragon yellow, V = soil + vermicompost, Bi = soil + biodigestate, VBi = soil + vermicompost + biodigestate, CT = contaminated soil without amendment (control).

Values followed by the same letter are not significantly different ($p > 0.05$). Small letters represent differences among PTE accumulation in each plant part in all treatments within the same species, whereas capital letters represent differences among PTE accumulation in each plant part in all plant species and all treatments.

et al., 2023). Cultivation of edible crops, particularly rice, must cease on such HM-contaminated land.

### 3.3. Cd and Zn accumulation in marigolds and their phytoremediation potential

Marigold organs of the Twenty yellow and Prime yellow varieties accumulated Zn in the following order: leaves > roots > flowers > stems, whereas Cd was accumulated as follows: roots > stems > leaves > flowers (Table 3). Dragon yellow, however, displayed a different tendency only in leaves, which had a somewhat higher Cd accumulation than stems. The combined vermicompost and biogestate significantly enhanced Zn and Cd accumulation in leaves (208.7 mg kg$^{-1}$ and 24.1 mg kg$^{-1}$, respectively) ($p < 0.05$). Furthermore, Dragon yellow, Twenty yellow, and Prime yellow all experienced substantial Zn and Cd accumulation from the vermicompost treatment (142.5–171.7 mg kg$^{-1}$ and 13.2–21.0 mg kg$^{-1}$, respectively). Moustakas et al. (2011) found that Zn concentration in marigold leaves increased to 373 mg kg$^{-1}$ in plants cultivated in soil contaminated with 15 mg kg$^{-1}$ Zn, and 205 mg kg$^{-1}$ in petals in soil contaminated with 15 mg kg$^{-1}$ Cd and 5 mg kg$^{-1}$ Zn. While cultivated in soil containing 15 mg kg$^{-1}$ Zn, leaves accumulated up to 373 mg kg$^{-1}$ Zn. In the current study, however, Zn accumulation in leaves and flowers was less pronounced. Cadmium accumulation in marigold leaves and flowers in all varieties from all treatments had lower concentrations, i.e., <1.2–4.9 times and 2.7–4.9 times, respectively, Cd accumulation where marigolds were grown in soil contaminated with comparable DTPA-Cd concentrations (32.6–68.4 mg kg$^{-1}$) (Lal et al., 2008). Marigold leaves and flowers are used extensively in many Asian and European countries, particularly India, Thailand, Denmark, and Germany, as important foods for humans and livestock, cosmetics, medicinal herbs, and ornamental plants. Many reports have addressed their potential for hyperaccumulating HMs and other pollutants (Biswal et al., 2021; Chauhan et al., 2022). Given that HMs are beneficial for pharmaceutical and food supplements for livestock and other biota including humans, it is imperative to investigate HMs in above-ground parts of marigolds in order to determine whether there is an associated health risk that exceeds the CODEX standard level of 0.4 mg kg$^{-1}$ for edible crops (Chunhabundit, 2016).

According to Morera et al. (2002), soil organic amendments are used for treatment of contaminated soils to stabilize or limit mobility of HMs in the rhizosphere; consequently, they can aid in the accumulation of HMs in roots, which ultimately leads to a successful phytostabilization strategy for excluders. In comparison to other marigold varieties in all amendment treatments, Prime yellow with vermicompost application enhanced Zn and Cd accumulation, with highest quantities in roots (121.3 mg kg$^{-1}$ and 65.7 mg kg$^{-1}$, respectively; $p < 0.05$). Several researchers have evaluated the potential of P-solubilizing bacteria for solubilizing organic forms of P by producing organic acids and reducing soil pH, thus enhancing P availability to plants (Alikhani et al., 2017; Sharma et al., 2013). Lowest soil pH was measured in the V treatment, which supports previous findings. However, a pH value of 7 in the V soil did not increase mobility of Cd or Zn. Precipitation of labile Cd and Zn, which lowers phytoavailability, occurs primarily in alkaline soil and at high P concentration (Bolan et al., 2003). Furthermore, P can form complexes with metals that have >6 linkages, which results in formation of metal phosphates (Bolan et al., 2003; Sneddon et al., 2006).

The most significant environmental factors that control Cd ion mobility are pH and oxidation-reduction potential. Cadmium is most mobile in soils at pH values at or below 5.0; in neutral and alkaline soil Cd is rather immobile. At pH values <8.0, it exists as the $Cd^{2+}$ ion. In addition, it may form complex ions such as $CdCl^{+}$, $CdHCO_3^{+}$, $CdCl_4^{2-}$, $Cd(OH)_3^{-}$, and $Cd(OH)_4^{2-}$ and organic chelates. Monovalent hydroxy ion species (e.g., $CdOH^{+}$) may occur that do not readily occupy cation exchange sites. In the alkaline pH range, $Cd^{2+}$ precipitates as $Cd(OH)_2$ and the less soluble $CdCO_3$. In strongly oxidizing environments Cd is likely to form minerals such as CdO and $CdCO_3$. Cadmium experiences weak adsorption to OM, silicate clays, and oxides unless pH is >6.0. At high concentrations, Cd complexes with humic substances or other organic molecules. Soil microbial activity is believed to influence the transformations of Cd in soils (Pichtel, 2019). Clay minerals, hydrous oxides, and pH are among the most important factors controlling Zn solubility in soil. Of lesser importance are precipitation as hydroxide, carbonate, and sulfide compounds, and complexation by OM. Zinc can enter some layer silicate clays (e.g., montmorillonite) and become immobilized. Soil OM is known to be capable of bonding Zn in stable forms (Kabata-Pendias, 2010). Because Zn is adsorbed by mineral and organic groups, its accumulation in surface horizons is fairly common (Pichtel, 2019).

Vermicompost is a domestic product that is widely used as a soil amendment in agricultural fields in Thailand. It is produced from various types of organic waste by composting with earthworms. Vermicomposting technology is cost-effective and eco-friendly, and provides OM and macronutrients to soil. Vermicomposting has the additional potential for amending HM-contaminated soil for phytoremediation (Iwai and Kruapukee, 2017; Jadia and Fulekar, 2008). By virtue of its high OM and nutrient content, vermicompost application enhances soil quality, organic carbon content, overall soil fertility, plant development, and soil microbial activity (Shen et al., 2022).

The bioconcentration factor for root (BCFR) for Zn was >1 and TF values for Zn were <1 for all marigold varieties (Table 4), suggesting that they are all excluders for Zn. The highest BCFR value for Zn was in the VBi treatment for Dragon yellow (9.6; $p < 0.05$). Bioconcentration factor for root values for Cd were generally <1, with the exception of

**Table 4**
Bioconcentration factor (BCF) and translocation factor (TF) for Cd and Zn ($n = 5$).

| Treatment | BCF for Cd | | BCF for Zn | | $TF_{Cd}$ | $TF_{Zn}$ |
|---|---|---|---|---|---|---|
| | Shoot | Root | Shoot | Root | | |
| PV | 1.3 ± 0.3aA | 0.6 ± 0.2aCD | 2.6 ± 0.3bBCD | 6.5 ± 1.7bcCDE | 2.1 ± 0.3aA | 0.4 ± 0.1bBCD |
| PBi | 1.3 ± 0.1aA | 0.6 ± 0.3aBCD | 3.3 ± 0.4aA | 8.8 ± 1aAB | 2.3 ± 0.8aA | 0.4 ± 0.0bCD |
| PVBi | 0.8 ± 0.1bCD | 0.3 ± 0.0bD | 2.3 ± 0.1bD | 4.8 ± 1.3cEF | 2.2 ± 0.3aA | 0.5 ± 0.1aA |
| PCT | 0.8 ± 0.1bCD | 0.4 ± 0.0bD | 2.0 ± 0.2cEF | 7.3 ± 1.5abBCD | 1.9 ± 0.2aAB | 0.3 ± 0.0cE |
| TV | 1.0 ± 0.1aB | 1.0 ± 0.2aABC | 2.3 ± 0.3bDE | 7.5 ± 2.1aBC | 1.1 ± 0.3aC | 0.3 ± 0.1aDE |
| TBi | 1.0 ± 0.1aBC | 0.9 ± 0.7aABC | 2.9 ± 0.4aBC | 7.2 ± 1.3aBCD | 1.3 ± 0.5aBC | 0.4 ± 0.1aBCD |
| TVBi | 0.7 ± 0.1bD | 0.5 ± 0.1aCD | 2.4 ± 0.3bD | 5.5 ± 1.6aDEF | 1.3 ± 0.2aC | 0.5 ± 0.1aABC |
| TCT | 0.6 ± 0.1bD | 0.5 ± 0.1aCD | 1.8 ± 0.3cF | 6.1 ± 2.4aCDE | 1.2 ± 0.4aC | 0.3 ± 0.1aDE |
| DV | 1.0 ± 0.2aD | 0.9 ± 0.2abABC | 2.5 ± 0.1bCD | 7.8 ± 0.2aABC | 1.1 ± 0.3aC | 0.3 ± 0.0bcDE |
| DBi | 1.0 ± 0.1aD | 1.2 ± 0.8aA | 3 ± 0.5aAB | 7.7 ± 1.9aABC | 1.1 ± 0.8aC | 0.4 ± 0.1bBCD |
| DVBi | 0.8 ± 0.1bD | 1.1 ± 0.5aAB | 2.4 ± 0.2bD | 9.6 ± 2aA | 0.8 ± 0.4aC | 0.3 ± 0.1cE |
| DCT | 0.5 ± 0.1cE | 0.4 ± 0.1bD | 1.7 ± 0.3cF | 3.7 ± 0.5bF | 1.3 ± 0.1aC | 0.5 ± 0.0aAB |

P = Prime yellow, T = Twenty yellow, D = Dragon yellow, V = soil + vermicompost, Bi = soil + biodigestate, VBi = soil + vermicompost + biodigestate, CT = contaminated soil without amendment (control). Values followed by the same letter are not significantly different ($p > 0.05$). Small letters represent differences among BCF or TF values in each plant part in all treatments within the same species, whereas capital letters represent differences among BCF or TF values in each plant part in all plant species and all treatments.

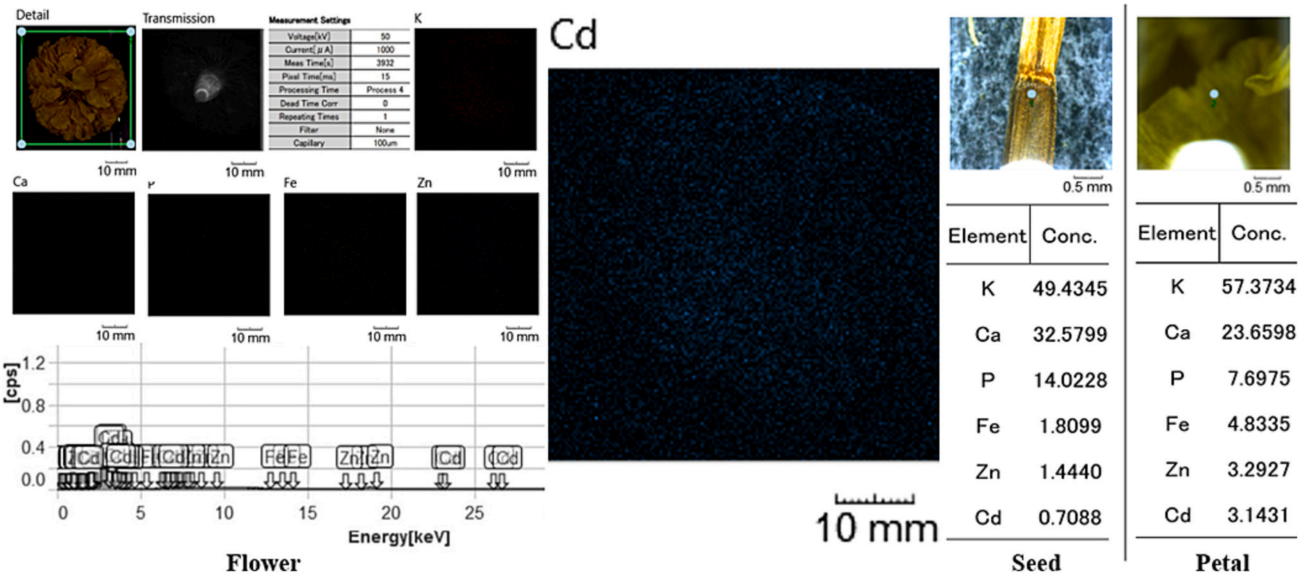
Twenty yellow in the V treatment (TF = 1), and in Dragon yellow in the VBi treatment (1.1). According to these findings, when vermicompost and biodigestate are applied to the contaminated soil, roots of Dragon yellow are largely capable of sequestering HMs within their tissue. This qualifies the Dragon yellow variety as suitable for phytostabilization in soil that is co-contaminated with Zn and Cd. Furthermore, the bioconcentration factor for shoot (BCFS) values for PTEs were >1, and TF values for PTEs were >1 of all tested plants in the V and Bi treatments, thus suggesting those plants accumulate substantial PTEs in shoots. In this study, none of the plants met the criteria for Zn and Cd hyperaccumulators, which requires that metals accumulate in shoots at levels of at least 10,000 mg $kg^{-1}$ and 100 mg $kg^{-1}$, respectively (Dai et al., 2022).

Previous studies (Boonyapookana et al., 2005; Pedron et al., 2009) have indicated that pH of soil contaminated with high quantities of HMs (i.e., Mn, Al) is a key factor that influences the dry biomass production of roots and shoots of a variety of plant species. This may result in reduced nutrient availability, high HM uptake, and eventual HM phytotoxicity. Nascimento et al. (2016) reported that many crops, including corn (*Zea mays* L.), and vetiver (*Vetiveria zizanioides* (L.) Nash) grown in acidic soils ($pH < 4$) contaminated with high concentrations of lead (Pb) experienced increased root collar diameter, plant height, and leaf structure, and pH adjustment improved those morphological traits. Shoots of castor bean (*Ricinus communis* L.) and sunflower (*Helianthus annuus* L.) species showed toxicity symptoms from high soil Pb concentrations, including chlorosis and ensuing necrosis of older leaves, as well as loss of the stem. In the current study, no overt phytotoxicity symptoms were observed after harvest.

No significant or slight differences were noted in percentage growth rate, in height and diameter of the marigold root collar, or in flower diameter between and within plants across all treatments ($p > 0.05$). Furthermore, no overt signs of toxicity in root or leaf traits were observed. This may indicate that marigolds have a high degree of adaptability and tolerance to harsh environments (Nascimento et al., 2016). The slightly alkaline soil in this study ($pH > 7$) also reduced phytotoxicity effects. Such soil exhibits lower Cd and Zn mobility, which in turn leads to lower bioavailability. Unaltered morphological traits of marigolds could potentially be linked with substantial N and P concentrations in the amendments, specifically those in the V and Bi treatments. These nutrients are necessary for normal synthesis and physiological metabolism. Additionally, they help plants to detoxify by synthesizing organic acids, certain proteins, and peptides that combine and complex with HMs (Shi et al., 2022).

The XRF spectroscopic results for K, Ca, P, Fe, Zn, and Cd across the entire flower surface of the marigold varieties under all treatments are shown in Fig. 1 and Table S1. Percentage element concentrations of the whole flower surface for all marigold varieties across all treatments were in the order: K > Ca > P > Fe > Zn > Cd. In this study, similar patterns of percentage element concentrations were detected. Potassium, a very mobile element, had the greatest and most even distribution throughout the flower surface; in contrast, the relatively immobile element Ca, showed much less coverage than K, by roughly 6.4 to 11.1 times. These findings corroborate those of a previous study which determined that K content predominated in edible flowers of *Chrysanthemum*, *Dianthus*, and *Viola* species (Rop et al., 2012). Potassium is the most abundant mineral element in plant tissues. For optimal growth, plants require a K concentration of 2–5 % of its dry weight, or 20–50 g $kg^{-1}$ d.m (Shaibur et al., 2008). Edible flowers are generally a rich source of macro- and microelements, with K, P, and Fe being particularly abundant. A small percentage of somewhat mobile elements (P) and immobile elements (Fe, Zn, and Cd) were detected on marigold flower surfaces. Zinc had greater percentage element concentrations on flower surfaces than did Cd, which was consistent with higher total Zn concentrations compared to total Cd concentrations present in whole flower tissue (Table 3). Cadmium is toxic and typically occurs in very small quantities in plants, whereas Zn is a micronutrient essential for optimal plant growth. Cadmium has no biological function and even in low quantities be harmful to plants (Dos Santos et al., 2020). The content of Zn in plant tissue is commonly >100 times that of Cd (Moustakas et al., 2011). These metals were accumulated in high quantities by flowers of *Minulus* × *hybridus* Magic Yellow and *M. didyma* (Zn), *Minulus* × *hybridus* Magic Red (Fe) and *Dianthus chinensis* Chianti (Cd) (Grzeszczuk et al., 2018).

Six elements from marigold flower parts—seeds and petals—were detected via X-ray spectroscopy, and elemental localization was determined (Tables S2 and S3). The order of percentage elemental composition of petals and seeds was: K > Ca > P > Fe > Zn > Cd, which was similar to the results of the surface of marigold flowers. The atomic percentages of K in marigold petals were higher than those for K in seeds across all marigold varieties and treatments. It was furthermore determined that the atomic percentages of P in seeds were higher than those in petals, and atomic percentages of Fe in petals were higher than those in seeds, especially in the Prime yellow and Twenty yellow varieties. The



| Element | Conc. |
|---|---|
| K | 49.4345 |
| Ca | 32.5799 |
| P | 14.0228 |
| Fe | 1.8099 |
| Zn | 1.4440 |
| Cd | 0.7088 |

| Element | Conc. |
|---|---|
| K | 57.3734 |
| Ca | 23.6598 |
| P | 7.6975 |
| Fe | 4.8335 |
| Zn | 3.2927 |
| Cd | 3.1431 |

**Fig. 1.** XRF spatial distribution of six elements and spectrum in the whole surface of marigold flower and the area target investigated (seed and petal of the flower) by XRF analysis.

**Table 5**
Antioxidant capacity (DPPH and FRAP, mg $mL^{-1}$) and bioactive compounds (TFC and TPC, mg $mL^{-1}$; chlorophyll and carotenoids, mg $L^{-1}$) of marigold flowers from each treatment ($n = 5$).

| Treatment | Antioxidant activity | | Bioactive compound | | | | | |
|---|---|---|---|---|---|---|---|---|
| | DPPH $IC_{50}$ | FRAP | TFC | TPC | Chlorophyll *a* | Chlorophyll *b* | Total chlorophyll | Carotenoids |
| PV | 80.2 ± 1.9cCD | 781.1 ± 63.6bHI | 24.4 ± 0.0dJ | 19.6 ± 0.8bF | 0.1 ± 0.0aBC | 0.1 ± 0.0aABC | 0.2 ± 0.1aABC | 29.1 ± 5.1bD |
| PBi | 77.6 ± 6.4cCDE | 995.6 ± 91.2bGH | 30.0 ± 0.1cH | 24.2 ± 0.6bF | 0.1 ± 0.1aAB | 0.2 ± 0.0aA | 0.3 ± 0.2aA | 44.2 ± 4.5aBC |
| PVBi | 124.2 ± 6.7bB | 1259.6 ± 242.3aEF | 39.4 ± 0.7bF | 34.4 ± 0.5aD | 0.1 ± 0.1aAB | 0.2 ± 0.0aAB | 0.3 ± 0.1aAB | 45.6 ± 12.1aB |
| PCT | 149.1 ± 13.4aA | 1488.3 ± 76.3aCDE | 53.8 ± 0.4aB | 32.1 ± 5.4aDE | 0.1 ± 0.0aABC | 0.2 ± 0.0aAB | 0.3 ± 0.1aABC | 46.6 ± 5.2aB |
| TV | 43.7 ± 3.5aC | 678.8 ± 343.7cI | 25.8 ± 0.1dI | 26 ± 0.5cEF | 0.1 ± 0.0aABC | 0.0 ± 0.0aD | 0.1 ± 0.1bC | 71.7 ± 5.1aA |
| TBi | 56.5 ± 2.5bG | 1143.1 ± 34.8bFG | 34.8 ± 0.3cG | 34 ± 10.6bcD | 0.1 ± 0.0aBC | 0.1 ± 0.0aABC | 0.2 ± 0.1abABC | 34.9 ± 6.2bCD |
| TVBi | 59.1 ± 7.5bFG | 1552.2 ± 89.7aCD | 49.4 ± 0.2aD | 50.2 ± 1.3aAB | 0.2 ± 0.1aA | 0.2 ± 0.0aABC | 0.3 ± 0.2aA | 74.0 ± 17.8aA |
| TCT | 47.6 ± 7.2bFG | 1329.2 ± 50.8abDEF | 44.7 ± 0.4bE | 38.3 ± 1.6bCD | 0.1 ± 0aABC | 0.2 ± 0.0aAB | 0.2 ± 0.2abABC | 64.2 ± 9.5aA |
| DV | 64.7 ± 1.4cDEF | 2066.5 ± 87.4aA | 64.1 ± 1.0aA | 50.9 ± 9.6aA | 0.1 ± 0aBC | 0.1 ± 0.0aBCD | 0.2 ± 0.1aABC | 41.6 ± 4abBC |
| DBi | 62.8 ± 1.5cEF | 1673.4 ± 75.7bBC | 53.4 ± 0.5bC | 42.7 ± 1.9abBC | 0.1 ± 0.0bC | 0.1 ± 0.0aD | 0.1 ± 0.1aBC | 36.8 ± 5.9abBCD |
| DVBi | 91.4 ± 6.2aC | 1827.3 ± 8.4bAC | 52.6 ± 0.0bcC | 39.7 ± 1.9bCD | 0.1 ± 0.0aBC | 0.1 ± 0.0aBCD | 0.2 ± 0.1aABC | 35 ± 2.8bCD |
| DCT | 76.9 ± 3.4bCDE | 1620.9 ± 205.1bBC | 52.1 ± 0.0cC | 42.9 ± 0.3abBC | 0.1 ± 0.0aBC | 0.1 ± 0.0aCD | 0.2 ± 0.1aABC | 43.1 ± 7.2aBC |

P = Prime yellow, T = Twenty yellow, D = Dragon yellow, V = soil + vermicompost, Bi = soil + biodigestate, VBi = soil + vermicompost + biodigestate, CT = contaminated soil without amendment (control), DPPH = DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity, FRAP = ferric reducing antioxidant power, TFC = total flavonoids content, TPC = total phenolics content.

Values followed by the same letter are not significantly different ($p > 0.05$). Small letters represent differences among antioxidant activity or bioactive compound values in flowers of all treatments within the same species, whereas capital letters represent differences among antioxidant activity or bioactive compound values in flowers of all plant species and all treatments.

seeds of all crops contain K, Ca, Fe, and Zn. These elements are necessary for seed germination, which enables a seedling to emerge and grow its first few leaves, even in the absence of additional nutrients (Hicsonmez et al., 2012). In the current study, the V treatment had the highest atomic percentage of Cd in petals of the Prime Yellow variety when all marigold varieties and treatments were taken into account ($p < 0.05$). This data corresponds with the finding that the same treatment had highest Cd concentration in whole flower tissue. The current findings demonstrate the effectiveness of XRF analysis to quantify HMs in plant matrices, as has been previously reported (Byers et al., 2019).

### *3.4. Effects of soil amendments on bioactive compounds and antioxidants in marigold flowers*

Vermicompost application resulted in highest levels of bioactive compounds for flowers of Dragon yellow (TPC, 50.9 mg $mL^{-1}$ and TFC, 64.1 mg $mL^{-1}$) ($p < 0.05$; Table 5) and antioxidant activity (FRAP, 2066.5 mg $mL^{-1}$) ($p < 0.05$; Table 5), while having modest $IC_{50}$ value (64.7 mg $L^{-1}$). Substantial quantities of FRAP, TFC, and TPC were detected in the VBi treatment, while the lowest $IC_{50}$ value was detected in the V treatment (43.7 mg $L^{-1}$, $p < 0.05$) for flowers of Twenty yellow. In the same treatments, flowers of Twenty yellow exhibited maximum carotenoid content (74 and 71.7 mg $mL^{-1}$, respectively) ($p < 0.05$). The low $IC_{50}$ contents were detected in the V and Bi treatments across all marigold varieties. Flower color, particularly yellow and orange, indicate a rich supply of antioxidants and a range of bioactive compounds, including carotenoids and flavonoids, which provide antioxidant capacity and protect against the damage induced by free radicals; therefore, flowers may be a useful indicator of plants that can adapt and survive well in harsh environments (Huang et al., 2022; Song et al., 2011).

Application of organic amendments has been shown in numerous studies to promote plant growth and development as well as improve soil quality. Additionally, employing optimal levels of organic amendments increases levels of antioxidants, bioactive compounds and enzymes in flowers (Abdolah zareh et al., 2013; Gonçalves et al., 2019; Singh et al., 2004). There is evidence that the humic substances present in vermicompost play a significant role in boosting biosynthesis of TPC in plants, which improves nutritional value and flavor of the plants and increases their ability to withstand harsh environments (Gholami et al., 2018; Theunissen et al., 2010). Increased Cd uptake and accumulation cause stress in plants, however. Reactive oxygen species (ROS) and lipid peroxidation have been shown to be indirectly increased by cadmium stress, which also decreases plant photosynthesis and antioxidant activities and interferes with uptake of essential nutrients. These factors ultimately contribute to impaired plant growth and development and ultimately cell death (Chen et al., 2021; Hassan et al., 2020). Growing hyperaccumulators (i.e., sunflower) on vermicompost-treated lead (Pb) and Cd-contaminated soils resulted in higher levels of antioxidant enzymes including catalase and superoxide dismutase in plant tissue (Mojdehi et al., 2021). Comparing bioactive compound contents and antioxidant activity for a Prime yellow variety of contaminated soil treated with vermicompost and biodigestate to the control treatment, however, showed no discernible effects. This phenomenon could be related to response by specific plant genotypes (Kubola et al., 2022).

Low quantities of total chlorophyll, chlorophyll *a* and chlorophyll *b* contents in flowers were recorded (0.1 to 0.2, 0.1 to 0.2 and 0.1 to 0.3 mg $L^{-1}$, respectively) (Table 5). This phenomenon is typically observed in the petals of different flowers. Plants are able to prevent accumulation of chlorophyll in their petals, which would mask the color of the flower. Furthermore, loss of chlorophylls during petal development is a key characteristic of flowering plants that makes them visually stand out against a background of leaves when flowers are ready to reward pollinators. On the other hand, leaves contain substantially higher chlorophyll concentrations as it is a necessary component of photosynthesis (Ohmiya et al., 2014). Marigold petals have relatively low chlorophyll content in comparison to other pigments such as betalains, carotenoids, and anthocyanins; as a result, as petals mature during flower development, their color changes (Tanaka et al., 2008). The results of the current study do not support recent findings that vermicompost application results in elevated carotenoid contents in plants (Shaabani et al., 2022) – carotenoids in the Prime yellow and Dragon yellow varieties in amended treatments were below those in the control, and there was no significant difference in carotenoid contents between the V and Bi treatments and the control treatment for Twenty yellow ($p > 0.05$). The Twenty yellow variety in the Bi treatment also had lower carotenoid content than the control ($p < 0.05$). Reduced levels of photosynthetic pigments such as carotenoids and chlorophylls may indicate HM stress (Feng et al., 2023). Substantially high values in the percentage growth rate in dry biomass across the V and VBi treatments for a Twenty Yellow variety are clearly consistent with substantial values in carotenoid concentrations. This implies that vermicompost and the combined vermicompost + biodigestate could improve plant growth and development, and production of bioactive compounds; however, other effects might alter antioxidant activities and production of bioactive compounds, including plant genotype, environmental conditions, culture techniques, and biotic and abiotic stresses (Yusof et al., 2018).

## 4. Conclusions

Soil amendments are important for improving growth performance and the production of marigold, particularly in terms of flower size and quantity. Quality marigold plants can generate substantial income for Thai farmers who cultivate them for commercial purposes. As demonstrated by the percentage growth rate in dry biomass in all marigold varieties across all amended treatments, biodigestate is regarded as a promising soil amendment for enhancing plant growth. The increase in flower numbers was influenced by all soil amendments, particularly the biodigestate treatment for the Prime yellow and Twenty yellow varieties. In this investigation, the Twenty yellow variety displayed appreciable growth performance in all amended treatments, while best performance was determined in the VBi treatment, which could indicate that HMs had minimal detrimental effects on plants. The production of bioactive chemicals from flower extracts increased when vermicompost was added to contaminated soils, particularly in the Dragon yellow, whereas increased DPPH free radical scavenging activity occurred in Twenty yellow varieties. When vermicompost was combined with biodigestate, dry biomass of a Twenty yellow variety increased, which is consistent with increased carotenoid contents. Furthermore, applying vermicompost to a Prime yellow variety increased quantities of Zn and Cd that accumulated in roots, which also increased the capacity for phytostabilization. Using XRF spectroscopy, a similar pattern was seen across all marigold varieties and treatments, revealing the presence of atomic percentages of Zn and Cd, respectively, across the whole flower surface, seeds and flower petals in descending order. The ICP-MS measurements of total Zn and total Cd values agree with this pattern. Marigolds have a strong potential for phytoremediation and bioindication of environmental damage, as demonstrated by differences in growth performance, HM accumulation, antioxidant capacity, and production of bioactive compounds. The application of vermicompost as a soil amendment has been shown to impact physicochemical properties of soils, including providing substantial amounts of nutrients, and enhancing soil pH, EC, and CEC. These factors may alter the antioxidant activities and production of bioactive compounds while also raising excluder potential of marigolds.

## CRediT authorship contribution statement

**Salinthip Chunwichit:** Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Theerawut Phusantisampan:** Writing – review & editing. **Alapha Thongchai:** Writing – review & editing. **Puntaree Taeprayoon:** Software. **Natthapong Pechampai:** Formal analysis. **Jittawan Kubola:** Writing – review & editing. **John Pichtel:** Writing – review & editing. **Weeradej Meeinkuirt:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, 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.

## Data availability

Data will be made available on request.

## Acknowledgements

The authors thank Ms. Tiyanun Jurkvon for assistance in operating the ICP-MS at the Mahidol University-Frontier Research Facility (MU-FRF), and Mr. Nawapol Udpuay, Dr. Suwilai Chaveanghong and Mr. Chawalit Takoon for the operation of the X-Ray fluorescence microscope. We are also grateful to Biosynthai Biotechnology Co., Ltd. for providing BioVis, a biodigestate product, for our experiments. This research project is supported by Mahidol University (Fundamental Fund: fiscal year 2023 by National Science Research and Innovation Fund (NSRF)).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.170791.

## References

Abdolah zareh, S., Fateh, E., Aynehband, A., 2013. Study effects of different sowing dates and chemical, organic and integrated fertilizer on grain active substance of *Silybum marianum* (L.) Gaerate. Iranian J. Med. Aromat. Plants 29 (2), 486–501.

Alaboudi, K.A., Ahmed, B., Brodie, G., 2018. Phytoremediation of Pb and Cd contaminated soils by using sunflower (*Helianthus annuus*) plant. Ann. Agric. Sci. 63, 123–127.

Alikhani, H.A., Hemati, A., Rashtbari, M., Tiegs, S.D., Etesami, H., 2017. Enriching vermicompost using P-solubilizing and N-fixing bacteria under different temperature conditions. Commun. Soil Sci. Plant Anal. 48 (2), 139–147.

Allen, S.E., Grimshaw, H.M., Parkinson, H.M., Quarmby, J.A., 1974. Chemical Analysis of Ecological Materials. Blackwell, Oxford.

Amouei, A.I., Yousefi, Z., Khosravi, T., 2017. Comparison of vermicompost characteristics produced from sewage sludge of wood and paper industry and household solid wastes. J. Environ. Health Sci. Eng. 15, 5. https://doi.org/10.1186/s40201-017-0269-z.

APHA, AWWA, WEF (American Public Health Association, American Water Works Association and Water Environment Federation), 2005. Standard Methods for Examination of Water and Wastewater. American Public Health Association, Washington, DC.

Aravindhan, S., Jawaharlal, M., Thamaraiselvi, S.P., Davamani, V., 2019. Effect of heavy metals on morphological and flowering parameters of African marigold (*Tagetes erecta*). Int. J. Chem. Stud. 7 (3), 4321–4323.

Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Anal. Biochem. 239 (1), 70–76.

Biswal, B., Singh, S.K., Patra, A., Mohapatra, K.K., 2021. Evaluation of phytoremediation capability of French marigold (*Tagetes patula*) and African marigold (*Tagetes erecta*) under heavy metals contaminated soils. Int. J. Phytoremediat. 24 (9), 945–954.

Black, G.R., 1965. Bulk Density: Method of Soil Analysis, Monograph No. 9 Part I. American Society of Agronomy Inc., Washington, DC.

Bolan, N.S., Adriano, D.C., Duraisamy, P., Mani, A., Arulmozhiselvan, K., 2003. Immobilization and phytoavailability of cadmium in variable charge soils. I. Effect of phosphate addition. Plant Soil 250, 83–94.

Boonyapookana, B., Parkpian, P., Techapinyawat, S., Delaune, R.D., Jugsujinda, A., 2005. Phytoaccumulation of lead by sunflower (*Helianthus annuus*), tobacco (*Nicotiana tabacum*), and vetiver (*Vetiveria zizanioides*). J. Environ. Sci. Health A 40, 117–137.

Bray, R.H., Kurtz, L.T., 1945. Determination of total, organic and available forms of phosphorus in soil. Soil Sci. 59, 39–45.

Byers, H.L., McHenry, L.J., Grundl, T.J., 2019. XRF techniques to quantify heavy metals in vegetables at low detection limits. Food Chem. X 1, 100001. https://doi.org/10.1016/j.fochx.2018.100001.

Chauhan, A.S., Chen, C.W., Singhania, R.R., Tiwari, M., Sartale, R.G., Dong, C.D., Patel, A.K., 2022. Valorizations of marigold waste for high-value products and their industrial importance: a comprehensive review. Resources 11 (10), 91. https://doi.org/10.3390/resources11100091.

Chen, L., Hu, W.F., Long, C., Wang, D., 2021. Exogenous plant growth regulator alleviate the adverse effects of U and Cd stress in sunflower (*Helianthus annuus* L.) and improve the efficacy of U and Cd remediation. Chemosphere 262, 127809. https://doi.org/10.1016/j.chemosphere.2020.127809.

Chintakovid, W., Visoottiviseth, P., Khokiattiwong, S., Lauengsuchonkul, S., 2008. Potential of the hybrid marigolds for arsenic phytoremediation and income generation of remediators in Ron Phibun District, Thailand. Chemosphere 70 (8), 1532–1537.

Chowdhury, A., Datta, R., Sarkar, D., 2018. Heavy metal pollution and remediation. In: Török, B., Dransfield, T. (Eds.), Green Chemistry. Netherlands, Elsevier, Amsterdam, pp. 359–373.

Chowdhury, S., Bolan, N., Farrell, M., Sarkar, B., Sarker, J., Kirkham, M.B., Hossain, M.Z., Kim, G.-H., 2021. Role of cultural and nutrient management practices in carbon sequestration in agricultural soil. In: Sparks, D.L. (Ed.), Advances in Agronomy. Academic Press, San Diego, pp. 131–196.

Chunhabundit, R., 2016. Cadmium exposure and potential health risk from foods in contaminated area, Thailand. Toxicol. Res. 32 (1), 65–72.

Ćirić, V., Prekop, N., Šeremešić, S., Vojnov, B., Pejić, B., Radovanović, D., Marinković, D., 2023. The implication of cation exchange capacity (CEC) assessment for soil quality management and improvement. Agric. For. 69 (4), 113–133.

Dai, H., Wei, S., Grzebelus, D., Skuza, L., Jia, J., Hou, N., 2022. Mechanism exploration of *Solanum nigrum* L. hyperaccumulating Cd compared to Zn from the perspective of metabolic pathways based on differentially expressed proteins using iTRAQ. J. Hazard. Mater. 440, 129717 https://doi.org/10.1016/j.hazmat.2022.129717.

Dhaliwal, S.S., Singh, J., Taneja, P.K., Mandal, A., 2020. Remediation techniques for removal of heavy metals from the soil contaminated through different sources: a review. Environ. Sci. Pollut. Res. 27, 1319–1333.

Dos Santos, M.L.S., De Almeida, A.F., Da Silva, N.M., Oliveira, B.R.M., Silva, J.V.S., Junior, J.O.S., Ahnert, D., Baligar, V.C., 2020. Mitigation of cadmium toxicity by zinc in juvenile cacao: physiological, biochemical, molecular and micromorphological responses. Environ. Exp. Bot. 179, 104201 https://doi.org/10.1016/j.envexpbot.2020.104201.

Enzmart Biotech, 2024. BioVis. https://enzmart.com/biovis.php. (Accessed 16 January 2024).

Feng, D., Wang, R., Sun, X., Liu, L., Liu, P., Tang, J., Zhang, C., Liu, H., 2023. Heavy metal stress in plants: ways to alleviate with exogenous substances. Sci. Total Environ. 897, 165397 https://doi.org/10.1016/j.scitotenv.2023.165397.

Fenglin, H., Ruili, L., Bao, H., Liang, M., 2004. Free radical scavenging activity of extracts prepared from fresh leaves of selected Chinese medicinal plants. Fitoterapia 75, 14–23.

Franzluebbers, A.J., 2020. Holding water with capacity to target porosity. Agric. Environ. Lett. 5, e20029 https://doi.org/10.1002/ael2.20029.

Gentili, R., Ambrosini, R., Montagnani, C., Caronni, S., Citterio, S., 2018. Effect of soil pH on the growth, reproductive investment and pollen allergenicity of *Ambrosia artemisiifolia* L. Front. Plant Sci. 9, 1335. https://doi.org/10.3389/fpls.2018.01335.

Gholami, H., Saharkhiz, M.J., Fard, F.R., Ghani, A., Nadaf, F., 2018. Humic acid and vermicompost increased bioactive components, antioxidant activity and herb yield of Chicory (*Cichorium intybus* L.). Biocatal. Agric. Biotechnol. 14, 286–292.

Gonçalves, J., Júnior, J.C.F.B., Carlos, L.D.A., Silva, A.P.C.M., De Souza, F.A., 2019. Bioactive compounds in edible flowers of garden pansy in response to irrigation and mycorrhizal inoculation. Rev. Ceres, Viçosa 66 (6), 407–415.

Grzeszczuk, M., Stefaniak, A., Meller, E., Wysocka, G., 2018. Mineral composition of some edible flowers. J. Elem. 23 (1), 151–162.

Haque, M.A., Sattar, M.A., Islam, M.R., Hashem, M.A., Khan, M.K., 2013. Evaluation of phosphate solubilizing bacteria in relation to phosphorus solubilization and phosphatase activity. Bangladesh J. Nucl. Agric. 29, 73–84.

Hassan, M.J., Raza, M.A., Rehman, S.U., Ansar, M., Gitari, H., Khan, I., Wajid, M., Ahmed, M., Shah, G.A., Peng, Y., Li, Z., 2020. Effect of cadmium toxicity on growth, oxidative damage, antioxidant defense system and cadmium accumulation in two Sorghum cultivars. Plants 9 (11), 1575. https://doi.org/10.3390/plants9111575.

Hegade, R.R., Chethanakumara, M.V., Krishnamurthy, S.V.B., 2023. Influence of soil organic carbon, water holding capacity, and moisture content on heavy metals in rice paddy soils of Western Ghats of India. Water Air Soil Pollut. 234, 192. https://doi.org/10.1007/s11270-023-06186-y.

Herawati, A., Mujiyo, Syamsiyah, J., Baldan, S.K., Arifin, I., 2020. Application of soil amendments as a strategy for water holding capacity in sandy soils. IOP Conf. Ser. Earth Environ. Sci. 724, 012014 https://doi.org/10.1088/1755-1315/724/1/012014.

Hicsonmez, U., Ozdemir, C., Cam, S., Ozdemir, A., Erees, F.S., 2012. Major-minor element analysis in some plant seeds consumed as feed in Turkey. Nat. Sci. 4, 298–303.

Hossen, M.A., Chowdhury, A.I.H., Mullick, M.R.A., Hoque, A., 2021. Heavy metal pollution status and health risk assessment vicinity to Barapakuria coal mine area of Bangladesh. Environ. Nanotechnol. Monit. Manag. 16, 100469 https://doi.org/10.1016/j.enmm.2021.100469.

Huang, X., Gao, W., Yun, X., Qing, Z., Zeng, J., 2022. Effect of natural antioxidants from marigolds (*Tagetes erecta* L.) on the oxidative stability of soybean oil. Molecules 27 (9), 2865. https://doi.org/10.3390/molecules27092865.

Ikeura, H., Kawasaki, Y., Kaimi, E., Nishiwaki, J., Noborio, K., Tamaki, M., 2016. Screening of plants for phytoremediation of oil-contaminated soil. Int. J. Phytoremediat. 18 (5), 460–466.

Iwai, C.B., Kruapukee, A., 2017. Using vermitechnology in soil rehabilitation for rice production in salt-affected area of Northeast Thailand. Int. J. Environ. Rural Dev. 8 (2), 88–93.

Jadia, C.D., Fulekar, M.H., 2008. Phytoremediation: the application of vermicompost to remove zinc, cadmium, copper, nickel and lead by sunflower plant. Environ. Eng. Manag. J. 7 (5), 547–558.

Kabata-Pendias, A., 2010. Trace Elements in Soil and Plants, fourth ed. CRC Press, Boca Raton, FL.

Kaur, M., Bhat, A., Sadik-Zada, E.R., Sharma, R., 2022. Productivity analysis and employment effects of marigold cultivation in Jammu, India. Horticulturae 8 (3), 263. https://doi.org/10.3390/horticulturae8030263.

Köksal, N., Yasemin, S., Özkaya, A., 2017. The effects of photoperiodism on growth and flowering of marigold (*Tagetes erecta*). Turkish J. Agri. Food Sci. Technol. 5 (10), 1189–1193.

Kubola, J., Chumroenphat, T., Pichtel, J., Meeinkuirt, W., 2022. Effects of soil amendments on metal uptake, antioxidant activities and production of bioactive compounds by sunflower sprouts. Sains Malays. 51 (2), 495–505.

Lal, K., Minhas, P.S., Shipra, Shipra, Chaturvedi, R.K., Yadav, R.K., 2008. Extraction of cadmium and tolerance of three annual cut flowers on Cd-contaminated soils. Bioresour. Technol. 99, 1006–1011.

Lee, M.E., Steiman, M.W., St. Angelo, S.K., 2020. Biogas biodigestate as a renewable fertilizer: effects of digestate application on crop growth and nutrient composition. Renew. Agric. Food Syst. 36 (2), 173–181.

Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol. 148, 350–382.

Lu, H., Wu, Y., Liang, P., Song, Q., Zhang, H., Wu, J., Wu, W., Liu, X., Dong, C., 2020. Alkaline amendments improve health of soils degraded by metal contamination and acidification: crop performance and soil bacterial community response. Chemosphere 257, 127309. https://doi.org/10.1016/j.chemosphere.2020.127309.

MEF (Ministry of the Environment, Finland), 2007. Government Decree on the Assessment of Soil Contamination and Remediation Needs (214/2007, March 1, 2007).

Mojdehi, F., Taghizadeh, M., Baghaie, A.H., Changizi, M., Khaghani, S., 2021. Role of antioxidant enzymes and plant performances towards heavy metal stress in ornamental sunflower by vermicompost implementation. Eur. J. Hortic. Sci. 86 (1), 69–77.

Morera, M.T., Echeverría, J., Garrido, J., 2002. Bioavailability of heavy metals in soils amended with sewage sludge. Can. J. Soil Sci. 82, 433–438.

Moustakas, N.K., Akoumianaki-Ioannidou, A., Barouchas, P.E., 2011. The effects of cadmium and zinc interactions on the concentration of cadmium and zinc in pot marigold (*Calendula officinalis* L.). Aust. J. Crop. Sci. 5 (3), 277–282.

Nascimento, S.M.S.G., Souza, A.P., Lima, V.L.A., Nascimento, C.W.A., Nascimento, J.J.V. R., 2016. Phytoextractor potential of cultivated species in industrial area contaminated by lead. Rev. Bras. Cienc. Solo 40, e0140808. https://doi.org/10.1590/18069657rbcs20140805.

Ohmiya, A., Hirashima, M., Yagi, M., Tanase, K., Yamamizo, C., 2014. Identification of genes associated with chlorophyll accumulation in flower petals. PLoS ONE 9 (12), e113738. https://doi.org/10.1371/journal.pone.0113738.

Olorunfemi, I.E., Fasinmirin, J.T., Ojo, A.S., 2016. Modeling cation exchange capacity and soil water holding capacity from basic soil properties. Eurasian Soil Sci. 5 (4), 266–274.

Paul, J.L., Lee, C.I., 1976. Relation between growth of *Chrysanthemums* and aeration of various container media. J. Amer. Soc. Hort. Sci. 101 (5), 500–503.

Pedron, F., Petruzzelli, G., Barbafieri, M., Tassi, E., 2009. Strategies to use phytoextraction in very acidic soil contaminated by heavy metals. Chemosphere 75, 808–814.

Pichtel, J., 2019. Fundamentals of Site Remediation for Metal- and Hydrocarbon-contaminated Soils, third ed. Bernan Press, Rockville, MD.

Rocha, C.S., Rocha, D.C., Kochi, L.Y., Carneiro, D.N.M., dos Reis, M.V., Gomes, M.P., 2022. Phytoremediation by ornamental plants: a beautiful and ecological alternative. Environ. Sci. Pollut. Res. 29, 3336–3354.

Rop, O., Mlcek, J., Jurikova, T., Neugebauerova, J., Vabkova, J., 2012. Edible flowers—a new promising source of mineral elements in human nutrition. Molecules 17 (6), 6672–6683.

Ross, D.S., Matschonat, G., Skyllberg, U., 2008. Cation exchange in forest soils: the need for a new perspective. Eur. J. Soil Sci. 59, 1141–1159.

Saengwilai, P., Meeinkuirt, W., Phusantisampan, T., Pichtel, J., 2020. Immobilization of cadmium in contaminated soil using organic amendments and its effects on rice growth performance. Exp. Health 12, 295–306.

Shaabani, M., Iriti, M., Mortazavi, S.N., Amirmohammadi, F.Z., Zamanian, K., 2022. The effects of two organic fertilizers on morpho-physiological traits of marigold (*Calendula officinalis* L.). S. Afr. J. Bot. 148, 330–335.

Shaibur, M.R., Shamim, A.H.M., Kawai, S., 2008. Growth response of hydroponic rice seedlings at elevated concentrations of potassium chloride. J. Agric. Rural Dev. 6 (1 & 2), 43–53.

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H., Gobi, T.A., 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. SpringerPlus 2 (1), 587. https://doi.org/10.1186/2193-1801-2-587.

Shen, Z., Yu, Z., Xu, L., Zhao, Y., Yi, S., Shen, C., Wang, Y., Li, Y., Zuo, W., Gu, C., Shan, Y., Bai, Y., 2022. Effects of vermicompost application on growth and heavy metal uptake of barley grown in mudflat salt-affected soils. Agronomy 12, 1007. https://doi.org/10.3390/agronomy12051007.

Shi, H., Wang, Y., Chen, H., Deng, F., Liu, Y., Cao, G., 2022. Changes in metal distribution, vegetative growth, reactive oxygen and nutrient absorption of *Tagetes patula* under soil cadmium stress. Horticulturae 8, 69. https://doi.org/10.3390/horticulturae8010069.

Singh, S., Saxena, R., Pandey, K., Bhatt, K., Sinha, S., 2004. Response of antioxidants in sunflower (*Helianthus annuus* L.) grown on different amendments of tannery sludge: its metal accumulation potential. Chemosphere 57 (11), 1663–1673.

Sneddon, I.R., Orueetxebarria, M., Hodson, M.E., Schofield, P.F., Valsami-Jones, E., 2006. Use of bone meal amendments to immobilise Pb, Zn and Cd in soil: a leaching column study. Environ. Pollut. 144 (3), 816–825.

Song, L., Wang, X., Zheng, X., Huang, D., 2011. Polyphenolic antioxidant profiles of Yellow Camellia. Food Chem. 129, 351–357.

Srisawat, L., Satapanajaru, T., Anurakpongsatorn, P., Jarusutthirak, C., Yoo-iam, M., 2021. Agricultural land suitability mapping for rice cultivation in severely heavy metal-contaminated land: case study of Mae Tao in Thailand. Water Air Soil Pollut. 232, 460. https://doi.org/10.1007/s11270-021-05385-9.

Taeprayoon, P., Printarakul, N., Somtrakoon, K., Chunwichit, S., Yooma, K., Wiangdao, S., Avakul, P., Meeinkuirt, W., 2023. Potentially toxic element accumulation of bryophyte taxa in contaminated soils at Tak Province, Thailand. Ecol. Indic. 147, 109971 https://doi.org/10.1016/j.ecolind.2023.109971.

Tanaka, Y., Sasaki, N., Ohmiya, A., 2008. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. Plant J. 54, 733–749.

Theunissen, J., Ndakidemi, P.A., Laubscher, C.P., 2010. Potential of vermicompost produced from plant waste on the growth and nutrient status in vegetable production. Int. J. Phys. Sci. 5 (13), 1964–1973.

Thongchai, A., Meeinkuirt, W., Taeprayoon, P., Pichtel, J., 2019. Soil amendments for cadmium phytostabilization by five marigold cultivars. Environ. Sci. Pollut. Res. 26, 8737–8747.

Thongchai, A., Meeinkuirt, W., Taeprayoon, P., Chelong, I., 2021. Effects of soil amendments on leaf anatomical characteristics of marigolds cultivated in cadmium-spiked soils. Sci. Rep. 11, 15909 https://doi.org/10.1038/s41598-021-95467-9.

Tinrat, S., 2016. Antioxidant activities and total phenolic content of multi-colored fruits and vegetables in Thailand. KKU Res. J. 21 (1), 1–11.

Tork, D.G., Anderson, N.O., Wyse, D.L., Betts, K.J., 2022. Ideotype selection of perennial flax (*Linum* spp.) for herbaceous plant habit traits. Agronomy 12 (12), 3127. https://doi.org/10.3390/agronomy12123127.

Walkley, A., Black, C.A., 1934. An examination of degradation method for determining soil organic matter: a proposed modification of the chromic acid titration method. Soil Sci. 37, 29–35.

Wang, F., Zhang, S., Cheng, P., Zhang, S., Sun, Y., 2020. Effect of soil amendments on heavy metal immobilization and accumulation by maize grown in a multiple-metal-contaminated soil and their potential for safe crop production. Toxics 8 (4), 102. https://doi.org/10.3390/toxics8040102.

Woraharn, S., Meeinkuirt, W., Phusantisampan, T., Avakul, P., 2021. Potential of ornamental monocot plants for rhizofiltration of cadmium and zinc in hydroponic systems. Environ. Sci. Pollut. Res. 28, 35157–35170.

Wuana, R.A., Okieimen, F.E., 2011. Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. Ecology, 402647. https://doi.org/10.5402/2011/402647.

Younis, A., Riaz, A., Qasim, M., Mansoor, F., Zulfiqar, F., Tariq, U., Ahsan, M., Naseem, M.K., Bhatti, Z.M., 2018. Screening of marigold (*Tagetes erecta* L.) cultivars for drought stress based on vegetative and physiological characteristics. Int. J. Food Allied Sci. 3 (2), 56–63.

Yu, H., Li, C., Yan, J., Ma, Y., Zhou, X., Yu, W., Kan, H., Meng, Q., Xie, R., Dong, P., 2023. A review on adsorption characteristics and influencing mechanism of heavy metals in farmland soil. RSC Adv. 13, 3505–3519.

Yusof, Z., Ramasamy, S., Mahmood, N.Z., Yaacob, J.S., 2018. Vermicompost supplementation improves the stability of bioactive anthocyanin and phenolic compounds in *Clinacanthus nuthans* Lindau. Molecules 23 (6), 1345. https://doi.org/10.3390/molecules23061345.