Phytoaccumulation of Zinc and its associated impact on the growth performance and tolerance index of six bioenergy crop plants grown in Zn-contaminated soil

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Research Article

Keywords: Zinc, bioenergy crops, phytoremediation, tolerance index, bioaccumulation

Posted Date: February 28th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1273917/v1>

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Phytoaccumulation of Zinc and its associated impact on the growth performance and tolerance index of six bioenergy crop plants grown in Zn-contaminated soil

Abstract

Crop plant remediation and reclamation of Zn-contaminated soils may pose a serious threat to food safety and thus human health. As a result, the current study was carried out to assess the capability of six bioenergy crop plants (BCP): *Zea mays* hybrid 360, *Z. mays* hybrid 123, *Helianthus annuus*, *Brassica juncea*, *Ricinus communis*, and *Simmondsia chinensis* to remediate and restore Zn-contaminated soils. The investigated plants can tolerate 150 mg kg⁻¹ Zn content of the soil, where they had tolerance index (TI) > 1 for all growth criteria, except the root dry weight (DW) of *S. chinensis*. *Z. mays* T123 and *R. communis* were the most susceptible plants, while *B. juncea* and *S. chinensis* were moderately tolerant, and *H. annuus* was the most tolerant to high Zn-concentrations. Increasing the soil Zn-content led to a significant ($p < 0.05$) increase in the concentration of Zn in the different tissues of the six BCP. The study BCP did not translocate Zn to their grains/seeds, and consequently they can be used safely in Zn phytoremediation from Zn-contaminated soils. The root and shoot Zn-content was negatively correlated with the TI of the root and shoot length and weight, while the TF of Zn from root to shoot was significantly proportional to the TI of the root length and weight. According to phytoremediation efficiency, the six study BCP were arranged as follow: *B. juncea* (31.9%) > *Z. mays* T123 (31.1%) > *Z. mays* T360 (27.6%) > *H. annuus* (20.8%) > *S. chinensis* (20.3%) > *R. communis* (15.3%). All tested BCP had high Zn concentrations in their roots and shoots, a high Zn uptake potential, and a high biomass at 150–450 mg kg⁻¹ Zn treatments, indicating that these plants are good candidates for the implementation of new strategy of cultivating BCP for phytoremediation in Zn-contaminated soils.

Keywords: Zinc, bioenergy crops, phytoremediation, tolerance index, bioaccumulation

Introduction

Heavy metal pollution is a serious problem in most countries around the world, and it can be caused by both natural and anthropogenic processes (Lajayer et al. 2019). Over the last few decades, urbanization and rapid industrialization have led to a significant increase in soil contamination with heavy metals like zinc (Bolan et al. 2014). Metal pollutants occur in soils in various forms and can pose a threat to soil components leading to soil degradation, a reduction in crop growth by interfering with their enzymatic reactions, photosynthetic activity, and nutrient homeostasis (Wei et al. 2021). These metals can also accumulate in the human body via the food chain, causing a variety of health issues such as nervous system disorders, brain, kidney, and liver damage, cardiomyopathy, cancer, and endocrine, nervous, and immune system impairments (Rai et al. 2019; Hu et al. 2020).

Zinc is an essential micronutrient for terrestrial plant growth and development, and it is the second most abundant and common metal in living organisms (Sidhu 2016). It plays a vital role in various physiological processes, including nucleic acid, lipid, and carbohydrate metabolism; enzyme activation; protein synthesis; and reproductive development (Grassi et al. 2020; Li et al. 2022). Nevertheless, Zn polluted soil is considered a major concern for global crop production (Miretzky et al. 2004). It is commonly found in high concentrations in wastewater from various industries, particularly mining, fertilizer industries, batteries, metal galvanization, pesticides, municipal sewage, and tire wear from automobile traffic (Rauch and Pacyna 2009; Dmuchowski et al. 2014). The main issue with Zn is its non-biodegradability and dispersion through an ecosystem's various trophic levels, as well as its persistence in nature (Elleuch et al. 2021). Under such circumstances, high content of Zn accumulates in plant tissues and can reach to toxic concentration ($> 300 \text{ mg kg}^{-1}$), which cause growth inhibition and physiological changes by reducing the contents of essential elements like Mn, Cu, and Fe (Connolly et al. 2003; Sagardoy

et al. 2009). Numerous physiological processes could be affected as a result of a high concentration of Zn such as altering photosynthetic activity, damaging oxidative membranes, inhibiting the synthesis of photosynthetic pigments, and electron transport (Vassilev et al. 2007; Yahaghi et al. 2019).

Many *in-situ* and *ex-situ* methods for removing metals from contaminated soil have been developed, including chemical or electrochemical precipitation, membrane separation processes, adsorption on activated carbon, reverse osmosis, and electrodialysis (Gong et al. 2018; Elleuch et al. 2021). Although these techniques provide efficient soil treatment, they are frequently time-consuming, expensive, destroy soil fertility, and are inappropriate for treating heavily contaminated soils (Liu et al. 2018). To avoid excessive accumulation of Zn in plants in contaminated areas, and thus in the food chain, soil remediation technologies such as phytoremediation, which are both inexpensive and least invasive to the environment, must be used (Yahaghi et al. 2019). Phytoremediation is a low-cost and environmentally friendly reclamation method that employs plant species that can absorb and accumulate metals in their vegetative tissue (Liu et al. 2018). The effectiveness of this technique is directly related to plants' ability to accumulate metal and produce biomass (Sarwar et al., 2017; Napoli et al. 2019). Cultivated plants exhibit varying degrees of tolerance to Zn deficiency or toxicity, depending on climatic and edaphic factors, plant species, exposure time, and metal concentration in the soil (Grassi et al. 2020).

Crop plant remediation and reclamation of Zn-contaminated soils may pose a serious threat to food safety and thus human health (Ramakrishna and Rao 2013; Sidhu et al. 2020). Thus, the reclamation and restoration of Zn-contaminated soils through the use of BCP is critical. Besides, it is essential to incorporate BCP with phytoremediation to achieve low-cost soil removal of

contaminants through biodiesel production. In this context, the current study was conducted to to:
i) assess the phytoremediation efficiency and tolerance of six investigated BCP: *Zea mays* (maize, hybrid 360), *Z. mays* (maize, hybrid 123), *Helianthus annus* (sunflower), *Brassica juncea* (indian mustard), *Ricinus communis* (castor oil), *Simmondsia chinensis* (jojoba) grown in Zn-contaminated soil, ii) estimate the concentration of Zn in the different plant organs and its impact on the morphological and growth metrics of the target plants. The outcome of this work is expected to afford an insight for using tested oil crop plants and detecting the most promising species in restoration of Zn-polluted soils.

Materials and Methods

Plant materials

Seeds of *Zea mays* (trihybrid 360), *Zea mays* (trihybrid 123), *Helianthus annus*, *Brassica juncea*, *Ricinus communis*, *Simmondsia chinensis* were identified and obtained from Agricultural Research Center (ARC), Giza, Egypt. Voucher specimens of the study harvested plants were identified by the authors and deposited in Umm Al-Qura University Herbarium, Makkah, Saudi Arabia.

Experimental design

A pot experiment was carried out from November 2018 to March 2019 in the greenhouse of the Faculty of Applied Science, Umm Al-Qura University, Saudi Arabia (21°18'56.3"N 39°56'47.8"E). The greenhouse settings were set at 20-28 °C temperatures and 50.0±4.0% relative humidity. The experiment was designed to assess Zn-uptake by six BCP cultivated in Zn-polluted soil. The soil was collected from cultivated fields and sands (2:1) from the neighborhood, air dried, sieved, and homogenized. This soil was characterized by 7.75 pH, and 420.0 µS cm⁻¹ electrical conductivity (EC). The total and DTPA-Zn (available) background levels were 35.6±3.5 and 20.7±5.8 mg kg⁻¹, respectively. Five different treatments of Zn as zinc sulphate (ZnSO₄·7H₂O)

were added to the soil corresponding to 0, 150, 300, 450, and 600 mg Zn kg⁻¹ dry soil. The Zn levels of 450 and 600 mg kg⁻¹ were selected depending on the phytotoxicity threshold documented by Alloway (2008). The treated soil was equilibrated for about 30 days, and then used in the experiment. The experiment used a randomized complete block design with 180 pots (6 crop plants, 5 treatments, and 6 replicates). Each pot (30 cm in diameter and 35 cm in height) was filled with 17 kg soil, and 5 grains/seeds of each plant were sown in each pot and left to grow in the greenhouse under natural day/light conditions for 100 days. After 50 days of sowing, the pots were drip irrigated twice a week, and the plants were thinned to 2 plants per pot (*R. communis* and *S. chinensis*) and 3 plants per pot (*Z. mays* T360 and T123, *H. annuus*, and *B. juncea*), while the emerged weeds were manually weeded.

Growth measurements

After 100 days from sowing, the investigated crop plants were harvested, and separated into stem, leaves, floral disk (*H. annuus*)/pod (*B. juncea*)/cob (*Z. mays*), and seeds/grains. The shoot and root length was measured for each plant, in addition to the cove length of *Z. mays* and floral disc diameter of *H. annuus* were also measured. Moreover, the different parts of each plant were weighed before and after oven-drying at 65 °C till constant weight to determine their fresh and dry weight (g/plant), respectively.

Plant analysis

Three samples from each harvested plant part from each treatment ($n = 60$ for *Z. mays*, *H. annuus* and *B. juncea*, and $n = 30$ for *R. communis* and *S. chinensis*) were collected, oven-dried (at 65 °C), grinded in a metal-free plastic mill for homogeneity, and sieved through a 2 mm mesh. To determine Zn concentration (Allen 1989), a 1 g ground sample was digested with a 20 ml tri-acid mixture of HNO₃:H₂SO₄:HClO₄ (5:1:1, v/v/v) to determine Zn concentration using an atomic absorption spectrophotometer (Shimadzu AA-6300; Shimadzu Co. Ltd., Japan).

Soil analysis

Three soil samples collected prior to crop plant cultivation were air-dried and sieved through a 2 mm sieve. 1/5 (w/v) soil water extracts were prepared to determine pH with a pH meter Model 9107 BN, ORION type, and electrical conductivity (EC) with a conductivity meter 60 Sensor Operating Instruction Corning. Furthermore, the total Zn content of soil samples collected prior to cultivation was estimated using plant analysis procedures. According to Lindsay and Norvell (1978), the available Zn concentration of soil before and after harvesting of each plant (in each treatment) was extracted and measured using DTPA (diethylene triamine pentaacetate).

Data analysis

Tolerance index (TI)

The tolerance index (TI) of the six tested BCP to Zn was assessed based on the growth measurements, including root and shoot length and biomass. It was estimated according to the equation of Wu et al. (2018):

$$TI = \frac{\text{morphological measurement in each Zn-treatment}}{\text{morphological measurement in the control soil}} \quad (1)$$

Zn-uptake

The bioaccumulation factor (BF) is an appropriate method for modeling heavy metal uptake from soil to plant root, whereas the translocation factor (TF) evaluates heavy metal transfer from root to various plant aerial parts. They were calculated to determine the ability of the plants studied to absorb Zn from the soil and transport it to their shoots (Ghosh and Singh 2005). According to Galal et al. (2021a), it was calculated as follows:

$$BF = C_{\text{root}} / C_{\text{soil}} \quad (2)$$

$$TF_{\text{shoot}} = C_{\text{shoot}} / C_{\text{root}} \quad (3)$$

$$TF_{\text{grain/seed}} = C_{\text{grain/seed}} / C_{\text{root}} \quad (4)$$

Where, C_{soil} , C_{root} , C_{shoot} , and $C_{\text{grain/seed}}$ are the Zn concentration (mg kg^{-1}) in the soil, root, stem, and grain/seed, respectively.

Phytoremediation efficiency (PE)

The phytoremediation efficiency of a plant, which investigates the potential of this plant to remove a heavy metal from contaminated soil, was estimated according to Yuana et al (2019):

$$\text{Phytoremediation efficiency (\%)} = \frac{C_1 - C_2}{C_1} \times 100 \quad (5)$$

where C_1 and C_2 are the concentrations of Zn (mg kg^{-1}) of the soil before cultivation and after harvesting for each plant, respectively.

Statistical analysis

Before performing analysis of variance (ANOVA), the data of plant variables and Zn-treatment parameters were checked for normality of distribution and homogeneity of variance, and data were log-transformed as needed. The significance of variation in Zn-concentration in the different organs of the different study BCP was tested using two-way ANOVA. To assess the relationship between the Zn-tolerance index of the plants' growth measurements and the bioaccumulation criteria, including Zn concentration and the bioaccumulation factor, the simple linear correlation coefficient (r) was calculated. SPSS software was used for all statistical analyses (SPSS 2012).

Results

Soil Zn-content

The application of Zn-treatments (0, 150, 300, 450, 600 mg kg^{-1}) for the soil supporting the growth of the six BCP led to soil available Zn contents of 20.7, 58.73, 102.82, 130.24, and 185.95 mg kg^{-1} (**Table 1**). After plant harvesting, the Zn content of the soil supporting the growth of each plant was significantly reduced ($p < 0.05$). Compared to the soil Zn-contents before

cultivation, the soil of *R. communis* had the highest contents (19.9 and 88.5 mg kg⁻¹) at the control and 300 mg kg⁻¹ Zn-treatment; respectively, while that of *Z. mays* T123 had the lowest (19.9 and 88.5 mg kg⁻¹). Besides, the soil of *S. chinensis* recorded the highest Zn contents (108.9 and 151.8 mg kg⁻¹), while that of *Z. mays* T360 had the lowest (84.3 and 112.4 mg kg⁻¹) at 450 and 600 mg kg⁻¹, respectively. Moreover, the soil of *H. annuus* had the highest content (53.4 mg kg⁻¹), while that of *B. juncea* had the lowest (37.5 mg kg⁻¹) at 150 mg kg⁻¹ Zn-treatment compare to their corresponding soil before cultivation.

Effect of Zn-treatments on the morphological traits

The present results indicated that the morphological traits of the six study bioenergy crop plant BCP s significantly increased to a certain limit and then significantly declined by increasing Zn concentration in the soil (**Fig. 1**). It was found that *Z. mays* T123, *H. annuus*, *B. juncea*, *R. communis*, and *S. chinensis* had their maximum root length (26.0, 7.3, 10.9, 37.0 and 15.9 cm, respectively) at 150 mg kg⁻¹ Zn-treatment, while *Z. mays* T360 attained its longest root (31.0 cm) at 300 mg kg⁻¹. Besides, *Z. mays* T123, *B. juncea*, and *S. chinensis* had their highest shoot lengths (129.3, 105.7 and 30.1 cm) at 150 mg kg⁻¹, while *Z. mays* T360 and *H. annuus* attained their highest shoot lengths (216.0 and 146.3 cm) at 300 mg kg⁻¹, and *R. communis* (90.7 cm) at 450 mg kg⁻¹ Zn-treatment. Moreover, the six BCP showed the minimum root and shoot lengths at 600 mg kg⁻¹ Zn-treatment. Furthermore, the highest cob length (18.1 and 10.8 cm) for *Z. mays* T360 and *Z. mays* T123, respectively as well as the disk diameter (8.7 cm) for *H. annuus* were recorded at 150 mg kg⁻¹, while the lowest (10.8, 7.3 and 6.5 cm), respectively was recorded at 600 mg kg⁻¹ Zn-treatment.

Effect of Zn-treatments on the growth traits

The data of plant weight of the study BCP organs under different Zn-treatments showed that 150 mg kg⁻¹ Zn content in the soil was the most flourishing for these plants except the root weight of *S. chinensis*, which had its highest fresh (FW) and dry (DW) root weight (0.99 and 0.33 g plant⁻¹) in the control soil (**Fig. 2**). However, most of the measured growth criteria had their minimum values in soil contaminated with 600 mg kg⁻¹ Zn, except *R. communis* root and shoot DW (4.1 and 27.8 g plant⁻¹) at 450 and 0 mg kg⁻¹ Zn-treatment, respectively. *Z. mays* T360, *Z. mays* T123, *H. annuus*, and *B. juncea* had their highest root FW (12.9, 8.7, 4.4 and 0.5 g plant⁻¹), root DW (4.1, 6.6, 2.1, and 0.1 g plant⁻¹), shoot FW (71.4, 106.3, 571 and 14.3 g plant⁻¹), and shoot DW (23.9, 28.7, 9.7 and 3.6 g plant⁻¹) in 150 mg kg⁻¹ Zn-treated soil. Likewise, *Z. mays* T360, *Z. mays* T123, and *B. juncea* attained their highest cope/pod FW (16.0, 16.2, and 5.9 g plant⁻¹), and DW (9.1, 5.7, and 4.2 g plant⁻¹) at 150 mg kg⁻¹, while the lowest (8.0, 7.9, and 2.5 g plant⁻¹) and (2.4, 1.9, and 1.5 g plant⁻¹) was at 600 mg kg⁻¹ Zn-treatment. At the same treatments, *Z. mays* T360, *Z. mays* T123, *H. annuus*, and *B. juncea* reached its maximum (30.5, 29.1, 8.7, and 0.5 g plant⁻¹) and minimum (19.1, 18.3, 6.5, and 0.4 g plant⁻¹) 100 seeds weight.

Tolerance index (TI)

The investigated BCP, except *S. chinensis* root DW, can tolerate 150 mg kg⁻¹ Zn content of the soil, where they had TI greater than 1 for all growth criteria (**Table, 2**). Besides, some species can tolerate higher soil Zn content such as *Z. mays* T360 root and shoot length and shoot weight; and *Z. mays* T123 root DW; *R. communis* root length; and *S. chinensis* shoot length can tolerate 300 mg kg⁻¹ Zn contents; and *B. juncea* root length can tolerate 450 mg kg⁻¹, and its shoot length and root DW can tolerate 300 mg kg⁻¹. Moreover, *H. annuus* had high potential to tolerate Zn-contaminated soils with the highest TI (1.84, 1.79, 1.60 and 1.49), which decreased with increasing Zn contents compared with the other investigated species. It is worth noting that *Z. mays*

T123 and *R. communis* were the most susceptible plants, while *B. juncea* and *S. chinensis* were moderately tolerant and *H. annuus* was the most tolerant to high Zn-concentrations.

Plants Zn-concentration

The two-way analysis of variance (ANOVA II) of the Zn concentrations in the different plants' tissues (root, shoot, cope/floral disc/pod, and seeds/grains) indicated significant variations ($p < 0.05$) in the accumulation potential of the different plants, Zn-treatments, and their intercept (**Table 3**). It was clear that increasing the soil Zn-content led to a significant ($p < 0.05$) increase in the concentration of Zn in the different tissues of the six BCP. Whereas, the least Zn concentration was obtained at the control soil, while the highest was at 600 mg kg⁻¹ Zn-treatment for all plant tissues, except *H. annuus* shoot, which attained its highest content (227.8 mg kg⁻¹) at 450 mg kg⁻¹ Zn-treatment. Regarding the plant species grown at 600 mg kg⁻¹ Zn treated soil, *Z. mays* T123 accumulated significantly ($p < 0.05$) the highest concentrations of Zn (458.95, 464.20, 209.85, and 233.00 mg kg⁻¹) in its root, shoot, grains and cope, respectively. On the other hand, *B. juncea* had the lowest Zn concentration (227.34 and 130.90 mg kg⁻¹) in its root and seeds; respectively, *R. communis* (199.36 mg kg⁻¹) in its shoot, and *H. annuus* (173.95 mg kg⁻¹) in its floral disk.

Zn uptake

The statistical analysis (ANOVA II) indicated a significant variation in the BF of Zn from soil to plants' root, and the TF from root to shoot and from root to grains/seeds among the investigated plants grown under different Zn-treatments (**Table 4**). The six study plants showed potential accumulation of Zn from the soil to their root with BF_{root} greater than 1 at each Zn-treatment, except at the control soil for *Z. mays* T123, *B. juncea*, *R. communis*, and *S. chinensis*, where their BF did not exceed 1. The highest BF (2.84) was recorded for *Z. mays* T123 at 450 mg

kg⁻¹ Zn-treatment. On the other side, *R. communis* showed low transfer of Zn from its root to the shoot at all Zn-treatments, while *B. juncea* at all treatments except 300 mg kg⁻¹, with TF_{shoot} greater than 1. Besides, *Z. mays* T360, *Z. mays* T123, and *B. juncea* showed translocation of Zn from root to shoot at the control treatment (TF_{shoot} = 1.43, 1.40, and 1.22) and 150 mg kg⁻¹ (TF_{shoot} = 1.03, 1.62 and 1.52), respectively. In addition, *H. annuus* had TF_{shoot} (1.03) at the control treatment, while *R. communis* and *S. chinensis* did not transfer Zn from the root to the shoot with TF_{shoot} less than 1. The highest TF_{shoot} (1.62) was recorded for *Z. mays* T123 at 150 mg kg⁻¹ Zn-treatment. Furthermore, the transfer of Zn from the root to the plants' grains/seeds indicated that the study BCP did not translocate this element to their grains/seeds with TF_{grain/seed} less than 1 at all Zn-treatment.

Phytoremediation efficiency (PE)

The phytoremediation efficiency of the study BCP showed significant variation ($p < 0.05$) at the different Zn-treatments and the intercept between plants and treatments (**Fig. 3**). The PE of *Z. mays* T360, and *R. communis* increased with the increasing Zn content in the soil from 8.6% and 3.2% at the control treatment to reach its maximum (39.4% and 24.9%) at 450 mg kg⁻¹, and then started to decrease (37.5 and 22.0%) at 600 mg kg⁻¹ Zn-treatment, respectively. Besides, the PE of *Z. mays* T123 and *H. annuus* ranged between 24.7% and 9.1% at 150 mg kg⁻¹ and 33.3% and 28.6% at 450 mg kg⁻¹; respectively. However, *B. juncea* and *S. chinensis* had their maximum PE (36.0 and 24.1%) at 150 mg kg⁻¹, while the lowest (27.5 and 15.5%) at the control and 600 mg kg⁻¹ Zn-treatments, respectively. It is worth to note that *Z. mays* T360 had the highest PE, while *R. communis* had the lowest to remove Zn metal from Zn-contaminated soils. On the average, the six study BCP were arranged according to their PE as follow: *B. juncea* (31.9%) > *Z. mays* T123

(31.1%) > *Z. mays* T360 (27.6%) > *H. annuus* (20.8%) > *S. chinensis* (20.3%) > *R. communis* (15.3%).

Correlation analysis

The simple linear correlation coefficient between the Zn-tolerance index and the bioaccumulation characteristics of the six BCP (**Table 5**) indicated that the TI of the root and shoot length and root and shoot weight was negatively correlated with root Zn content ($r = -0.618$, -0.723 , -0.573 , and -0.722), shoot Zn-contents ($r = -0.483$, -0.666 , -0.362 , and -0.787). Besides, the shoot length TI was negatively correlated ($r = -0.257$) with the bioaccumulation of Zn from soil to the root. On the other hand, The TF of Zn from root to shoot was significantly proportional to the TI of the root length and root weight ($r = 0.258$ and 0.439 , respectively).

Discussion

Soil properties

Zinc is a significant ecotoxic metal that pollutes both the terrestrial and aquatic environments (Elleuch et al. 2021). The total soil Zn content used in the present investigation was 35.6 mg kg^{-1} , which did not exceed the range ($50\text{--}55$ and $10\text{--}100 \text{ mg kg}^{-1}$) reported by Kiekens (1995) and Mertens and Smolders (2013), respectively for normal soils. Besides, Kabata-Pendias and Pendias (2001) reported Zn content ($47\text{--}61 \text{ mg/kg}$) in natural medium loamy soil such as that used in the present study. Therefore, the soil used in the present investigation was Zn-uncontaminated, but after treatments it became Zn-contaminated. Moreover, available Zn to plants is affected by the total Zn content, pH, concentrations of other heavy metals, and macro-nutrients (Alloway 2008). In the present study, the available Zn of the soil, before the experiment, ranged between 20.7 and 185.9 mg kg^{-1} at the control and 600 mg kg^{-1} Zn-treatment, respectively of the slightly alkaline soil ($\text{pH} = 7.75$). According to Al-Sodany et al. (2022), slightly alkaline pH may limit metal availability and solubility in the soil, besides Liu et al. (2020) reported that soils with

a pH > 7.4 have relatively low available Zn content because the solubility of Zn decreases with increasing pH.

Plant Growth

Zinc is an essential trace element for proper plant growth and reproduction and plays an important role in many biological processes (Cakmak 2008, Noulas et al. 2018). The investigated BCP showed the highest root, shoot length, and cope/floral disc at 150 mg kg⁻¹ Zn-treatment, except *Z. mays* T360 root and shoot, and *H. annuus* shoot, which attained their highest values at 300 mg kg⁻¹, and *R. communis* shoot length at 450 mg kg⁻¹ Zn-treatment. In the same context, Sidhu et al. (2020) reported that when treated with 100, 250 mg kg⁻¹ Zn regimes, the root and shoot length of *Coronopus didymus* increased compared to the control. The six BCP, on the other hand, had the smallest morphological measurements after 600 mg kg⁻¹ Zn treatment. Owing to Galal et al. (2021b), heavy metals, including Zn, induce a number of morphological and physiological changes that inhibit the growth of most plant species. Several studies, including Farahat et al. (2017) on wheat and maize, Ahmed and Slima (2018) on jew's mallow, Galal et al. (2018) on cabbage, Shehata and Galal (2020) on cucumbers, Galal et al. (2021c) on castor oil, Galal et al. (2021a) on garden pea, and Ahmed et al. (2021) on okra plant, have reported decreased plant growth parameters like root and shoot length, plant biomass and production, in contaminated soils with heavy metals including Zn.

Zinc is required in small but critical concentrations for plant growth, and if available amounts are insufficient, plants will suffer from physiological stress caused by the dysfunction of several enzyme systems and other metabolic functions that require Zn (Alloway 2008). The study BCP organs showed that 150 mg kg⁻¹ soil Zn-treatment was the most flourishing for all plants except the root weight of *S. chinensis*, which had its highest fresh (FW) and dry (DW) weight in the control soil. In a similar study, Sidhu et al. (2020) discovered that a moderate Zn supply (100

- 250 mg kg⁻¹) in the soil increased *C. didymus* growth, whereas a 500 mg kg⁻¹ Zn regime reduced growth and biomass in comparison to the control plants. According to the current findings, the different organs of *B. juncea* reached their maximum weight at 150 mg kg⁻¹ Zn treatment and were then negatively affected ($p < 0.05$) by increasing soil Zn content. These findings were consistent with the those of Ebbs and Kochian (1998) and Grassi et al. (2020), who found that the highest DW of *B. juncea* was found in the control and then decreased as Zn content in the soil increased, indicating that Zn has a negative effect on the plant growth. Furthermore, Ashraf et al. (2019) observed on *H. annuus* plants that a low or moderate heavy metal dose can facilitate plant growth.

The majority of the measured growth criteria, on the other hand, had their minimum values in soil contaminated with 600 mg kg⁻¹ Zn, with the exception of *R. communis* root DW at 450 mg kg⁻¹ Zn-treatment. Excessive Zn application, according to Jin et al. (2008) and Michael and Krishnaswamy (2011), usually inhibits growth in non-hyperaccumulating plants such as *Sedum alfredii* and *Phaseolus vulgaris*, respectively. Grassi et al. (2020) discovered that the FW and DW of *R. communis* shoots and roots did not increase significantly ($p < 0.05$) with increasing soil Zn concentrations, which is consistent with the current study. Furthermore, Wang et al. (2016) discovered that increasing soil Zn concentrations up to 400 mg kg⁻¹ increased *R. communis* biomass, whereas Zn concentrations greater than 600 mg kg⁻¹ reduced plant growth in comparison to the control. Furthermore, Yadav et al. (2009) and Grassi et al. (2020) reported that soil Zn enhanced the growth of *Jatropha curcas*, a Euphorbiaceous species related to *R. communis*, up to 1000 mg kg⁻¹, while higher Zn concentrations stunted plant growth. One reason for decreased growth and biomass is likely due to excess Zn interfering with vital metabolic activities in plants (Michael and Krishnaswamy 2011) and nutrient uptake (Sidhu, 2016; Sidhu et al. 2020).

Plant-tolerance to Zn-treatments

Plants differ markedly in their sensitivity/tolerance to Zn concentration, where the most Zn-efficient plants are able to produce more biomass and production under conditions of low available Zn supply than Zn-inefficient plants (Alloway 2008). The present investigation revealed that *Z. mays* T123 and *R. communis* were the most susceptible plants, while *B. juncea* and *S. chinensis* were moderately tolerant and *H. annuus* was the most tolerant to high Zn-concentrations. Shi and Cai (2010) discovered, on the other hand, that among the eight studied species, *H. annuus* did not grow well under Zn stress (400–800 mg kg⁻¹). *Brassica* species have been reported by some authors (e.g., Barrameda-Medina et al., 2014; Mourato et al., 2015) to be accumulator plants with high tolerance to heavy metals in soils. Barrameda-Medina et al. (2014) also described an increase in Zn in leaves and stems as a result of *B. oleracea*'s tolerance mechanism when grown hydroponically in Zn-spiked solution. Furthermore, plant roots were more resistant to Zn stress than shoots, which could be attributed to a reduction in Zn translocation to the aerial parts (Kabata-Pendias and Pendias, 2001); or, more likely, to the development of mechanisms such as cellular compartmentalisation in the roots, enhancing plant resistance to metal stress (Leitenmaier and Küpper 2013).

Zn uptake by plants

Zinc accumulation in the different organs of the six BCP was dose-dependent since Zn concentration increased in the plant tissues in response to its increase in the soil. Similar results were reported by Wang et al. (2016), Akanbi-Gada et al. (2019) and Grassi et al. (2020), who found that the Zn concentration in *Solanum lycopersicum* and *B. juncea* plant tissues increased considerably ($p < 0.05$) compared to the control as the soil Zn-content increased. Furthermore, Hernandez-Viezcas et al. (2013) discovered dose-dependent Zn accumulation in soybean treated with various Zn concentrations. The root accumulated more Zn concentrations than the shoots in

the majority of the plants studied (except *B. juncea*). This finding was consistent with the findings of Liu et al. (2015), Jahan et al. (2018), and Akanbi-Gada et al. (2019), who attributed the low Zn translocation to shoots to Zn's ability to effectively bind to root tissues, or possibly due to the plant's exclusion strategy to avoid Zn toxicity. Similarly, Zhao et al. (2012) and Mukherjee et al. (2013) found similar low Zn translocation from the root to the shoot of maize and green pea plants. Grassi et al. (2020) discovered no significant difference in Zn accumulation in the roots and shoots of *B. juncea*. According to Marschner (1995), most plants have Zn concentrations ranging from 30 to 100 mg kg⁻¹, with concentrations above 300 mg kg⁻¹ considered toxic. As a result, the Zn concentrations in the above-ground parts of the investigated BCP (with the exception of *Z. mays* T123 and *B. juncea* at 600 mg kg⁻¹ Zn-treatment) were within the safe range.

Zn uptake

The BF exemplifies a plant species' ability to collect elements from the soil, and thus the properties of phytoremediation (Al-Sodany et al. 2022). It was reported that the amount of available metal in the soil influenced the transfer of heavy metals from soil to root (Li et al. 2022). The study BCP showed potential accumulation of Zn from the soil to their root with BF greater than 1 at each Zn-treatment, except at the control soil for *Z. mays* T123, *B. juncea*, *R. communis*, and *S. chinensis*, where their BF did not exceed 1. However, Grassi et al. (2020) found that the estimated BF value was < 1 for *R. communis* plants cultivated in Zn-spiked soil, while that cultivated in control was > 1. In addition, *B. juncea* showed transfer of Zn from its root to the shoot at all Zn-treatments, except 300 mg kg⁻¹ with TF_{shoot} higher than 1. Meanwhile, *R. communis* and *S. chinensis* showed low transfer of Zn from their root to the shoot at all Zn-treatments. According to Kabata-Pendias and Pendias (2001), high Zn content in the soil facilitates its translocation from the roots to the shoots, resulting in high Zn acclimatisation in the shoots, which corresponds to

greater Zn phytoextraction potential of *B. juncea*. This species has been considered a Zn-tolerant plant species capable of absorbing and translocating high Zn amounts in their tissues from high soil Zn content. Controversily, Grassi et al. (2020) recorded that the estimated BF and TF values for *B. juncea* and the TF for *R. communis* were less than 1 for the Zn-spiked soil suggesting the potential of these species in phytostabilization. Moreover, *Z. mays* T360 and *Z. mays* T123 above 150 mg kg⁻¹, and *H. annuus* above the control Zn-treatments had TF less than 1, suggesting their potential suitability for Zn phytostabilization. Similar results were suggested by Farahat et al. (2017) on *Z. mays* crop plants. It is worth to note that the study BCP did not translocate this element to their grains/seeds. Consequently, these species can be used safely in phytoremediation of Zn from Zn-contaminated soils.

Tolerance index-bioaccumulation characteristics correlations

The simple linear correlation coefficient between the Zn-tolerance index and the bioaccumulation characteristics of the six BCP indicated that the TI of the root and shoot length and root and shoot weight was negatively correlated with root and shoot Zn content. Our results coincided with those of Shi and Cai (2010), who recorded TI values with significant negatively correlation with the Zn concentration in the roots and shoots of some crop plants. They showed that the high Zn tolerance of hemp, flax, and rapeseed was due to lower Zn concentrations in the shoots due to the use of the exclusion strategy. On the other side, the TF of Zn from root to shoot was significantly proportional to the TI of the root length and root weight. Hamilton et al. (1993) and Han et al. (2011) discovered that soil DTPA-extractable Zn had a positive correlation with crop uptake in similar conditions, implying that it could be used as a reliable index for predicting Zn availability in high pH soils. Furthermore, Liu et al. (2020) discovered a strong relationship between soil DTPA-extractable Zn and Zn uptake by soybean and wheat.

Phytoremediation efficiency

The PE is determined by the amount of metal uptake from the contaminated soil by the plant tissues (Shi and Cai 2010). The ability of plants to extract large amounts of heavy metals from soil is critical to the development of phytoremediation technology (Yang et al. 2017; Zou et al. 2021). The Zn content of the soil supporting the growth of the six BCP was significantly reduced ($p < 0.05$) at the end of the experiment. At lower soil Zn-contents, *B. juncea*, and *Z. mays* T123 were the potential species for uptaking Zn from the soil, while at higher soil Zn-contents, *Z. mays* T360 was the most efficient. All tested BCP had high Zn concentrations in the roots and shoots, high BF and Zn uptake, and high biomass at 150–450 mg kg⁻¹ Zn treatments, indicating that these plants are good candidates for the implementation of our new strategy of cultivating BCP for phytoremediation in Zn-contaminated soils. Zhou et al. (2020) reported similar results on some crop plants, including *H. annuus*. According to Shi and Cai (2010), the ideal plant for phytoextraction should be tolerant to high levels of the metal and accumulate high levels of the metal in its harvestable parts, so *H. annuus* was a good candidate for Zn-phytoextraction. It is worth to note that *Z. mays* T360 had the highest efficiency, while *R. communis* had the lowest to remove Zn metal from Zn-contaminated soils. On the average, the six study BCP were arranged according to their PE as follow: *B. juncea* (31.9%) > *Z. mays* T123 (31.1%) > *Z. mays* T360 (27.6%) > *H. annuus* (20.8%) > *S. chinensis* (20.3%) > *R. communis* (15.3%). Although our results revealed the phytoremediation potential of the six investigated BCP, attention should be paid when extrapolating these pot study results to the field.

Conclusion

The study BCP showed that 150 mg kg⁻¹ soil Zn-treatment was the most flourishing for all plants growth metrics, while 600 mg kg⁻¹ Zn was the most diminishing treatment. *Z. mays* T123

and *R. communis* were the most susceptible plants, while *B. juncea* and *S. chinensis* were moderately tolerant and *H. annuus* was the most tolerant to high Zn-concentrations. The study BCP did not translocate Zn to their grains/seeds, and consequently they can be used safely in Zn phytoremediation from Zn-contaminated soils. The root and shoot Zn-content was negatively correlated with the TI of the root and shoot length and weight, while the TF of Zn from root to shoot was significantly proportional to the TI of the root length and weight. *Z. mays* T360 had the highest efficiency, while *R. communis* had the lowest to remove Zn metal from Zn-contaminated soils. At a moderate Zn supply (150–450 mg kg⁻¹), the tested BCP had high Zn concentrations in their roots and shoots, high BF and Zn uptake potential, and high biomass, indicating that these plants are good candidates for implementing our new strategy of cultivating BCP for phytoremediation in Zn-contaminated soils.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

Competing interests The authors declare no competing interests.

Funding

The authors would like to thank the Deanship of Scientific Research at Umm Al-Qura University for supporting this work by Grant Code: (22UQU4331100DSR01)".

Author contribution Conceptualization:

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Data curation: A.Q., M.E., H.O. and T.G.

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Writing-review and editing: T.G., M.E., and H.O.

All authors have read and agreed to the published version of the manuscript.

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Figures

Fig. 1. Morphological measurements of the six oil crop plants grown under different Zn treatments for 100 days.

Figure 1

Please See image above for figure legend.

Fig. 2. Dry weight standing crop (g/plant) of the different organs of the six oil crop plants grown under different Zn treatments for 100 days. FW: fresh weight, DW: dry weight.

Figure 2

Please See image above for figure legend.

Fig. 3. Phytoremediation efficiency (%) of the six oil crop plants grown under different Zn-treatments for 100 days. *Z. mays*: *Zea mays*; *H. annus*: *Helianthus annuus*; *B. juncea*: *Brassica juncea*; *R. communis*: *Ricinus communis*; *S. chinensis*: *Simmondsia chinensis*. ***; $P < 0.001$.

Figure 3

Please See image above for figure legend.

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