Zincum Metallicum, a homeopathic drug, alleviates Zn-induced toxic effects and promotes plant growth and antioxidant capacity in *Lepidium sativum* L

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Abstract
In this study, we investigated the effect of the homeopathic drug Zincum Metallicum (ZM) on zinc (Zn) toxicity in the plant species *Lepidium sativum* L. We focused on growth parameters, Zn uptake and numerous biochemical parameters. Seedlings were hydroponically subjected during 7 days to 0.05, 500, 1000, 1500 and 2000 µM Zn2+, in the absence or presence of 15ch or 9ch ZM. In the absence of ZM, Zn induced negative effect on growth especially at the dose of 2 mM. Zn induced also chlorosis, reduced total chlorophyll and/or carotenoid content and increased the level of malondialdehyde (MDA). Under Zn toxicity (500, 1000 and 1500 µM), the superoxide dismutase (SOD), catalase (CAT), gaiacol peroxidase (GPX) and glutathione reductase (GR) activities were increased or not significantly affected, while at 2000 µM Zn affected the activity of these enzymes. At the highest Zn level (2 mM), proline and total polyphenol and flavonoid contents were markedly increased in leaves and roots of *L. sativum*. Additionally, ZM supply considerably ameliorated the plant growth, photosynthetic pigment contents and increased non-enzymatic antioxidant molecules and enzymatic activities against Zn-induced oxidative stress. Our data suggest that homeopathic properties of ZM may be efficiently involved in the restriction of Zn-induced oxidative damages, by lowering Zn accumulation and translocation in the leaves and roots of *Lepidium sativum* L.

Keywords Antioxidant enzymes · Growth · *Lepidium sativum* L. · Polyphenols · Zinc · Zincum Metallicum

Abbreviations
H2O2 Hydrogen peroxide
ROS Reactive oxygen species
MDA Malondialdehyde
SOD Superoxide dismutase

CAT Catalase
GPx Glutathione peroxidase
GR Glutathione reductase
ZM Zincum Metallicum

Introduction
Zinc (Zn) is an indispensable micronutrient for growth and development of plants, but it is considered as a major industrial pollutant (Li et al. 2013; Wei et al. 2021). Under normal conditions, Zn is required in small amount to allow the important plant physiological, biochemical and molecular pathways including photosynthesis, nitrogen metabolism, chlorophyll and auxin synthesis, activation of enzymes (cofactors), DNA replication and transcription, and the control of gene expression (Rout and Das 2003; Rizwan et al. 2019). It plays a key role in maintaining membrane integrity and permeability, the protection of thiol groups of proteins and the inhibition of reactive oxygen species production (ROS) induced by transition
metals including iron and copper (Haleng et al. 2007). However, elevated Zn concentrations might inhibit plant growth by affecting plant physiological metabolisms (Gong et al. 2020; Tsonev and Lidon, 2012). In this circumstance, substantial data revealed that high Zn concentrations inhibited cell division and cell elongation of root system, decreased photosynthesis, disturbed mitochondrial structure and absorption and translocation of nutrients and induced the overproduction of ROS (Doillon, 2010; Rout and Das 2003; Tsonev et al. 2012). Excessive production of ROS in plant would, therefore, enhance oxidative stress and disrupt the redox homeostasis (Todeschini et al. 2011). For these reasons, many previous studies focused on elucidating the negative impacts of heavy metals on plant crops and improving their growth and productivity (Tsonev et al. 2012; Li et al. 2012; Dos Santos et al. 2019).

The application of homeopathy in agriculture, known as agricultural homeopathy, would be very effective in terms of plant tolerance to abiotic stress, particularly heavy metals. In fact, numerous studies have shown that potentiated homeopathic medicine can improve the physiological activities in numerous cultivated plants species, via modulating enzymatic activities, total sugar, protein and chlorophyll content (Mazón-Suástegeuí et al. 2019). For instance, the study of Banerjee and Sukul (2013) revealed that cuprum sul phuricum, a homeopathic drug, alleviated the toxic effect of copper though promoting seed germination and peroxidase activity in Vigna unguiculata L.

Garden cress (Lepidium sativum L.), an annual herbaceous species from the Brassicaceae family, is one of the well-known aromatic and medicinal plant (Vaishali and Neeta, 2014). It is of economic importance since it has been used as treatment of various diseases and the reduction of the effects of chemotherapy (Abo El Maati et al. 2016). Lepidium sativum L. is rich in bioactive molecules, including fatty acids, tocopherol, carotenoid, phytosterol, campesterol, avenasterol and phenolic compounds, to which the antimicrobial, antihypertensive, antioxidant, antispasmodic, antidiarrheal, antiasthmatic, hypoglycemic and hypolipidemic activities are attributed (Abo El Maati et al. 2016). Despite the fact that L. sativum have been studied for its tolerance to abiotic stress including salinity (Al-Sammarraie et al. 2020) and copper excess (Rombel-Bryzek et al. 2017), little is known about its response to zinc (Zn) contrast.

The current study aims to explore the effect of different Zn concentrations on the growth, physiological and biochemical parameters of L. sativum and evaluate the putative effect of the exogenous application of Zincum Metallicum (15 or 9 ch) on Zn-induced toxicity. We focused essentially on the growth parameters, chlorophyll, carotenoid content proline contents, lipid peroxidation and antioxidant system.

### Material and methods

#### Plant material and growth conditions

Seeds of L. sativum were collected from the region of Djerba (33° 48′ 27.353″ N 10° 50′ 42.529″ E). Seeds were sterilized with bleach solution (30% commercial bleach) for 15 min then washed 3 times with sterile water and germinated in Petri dishes containing two sheets of filter paper soaked with distilled water. Five days after germination, seedlings were transplanted into plastic pots containing 1.3 L of quarter-strength Hoagland nutrient solution continuously aerated and renewed every 7 days (Arnon and Hoagland 1940). Plant culture was maintained in a growth chamber under 22 °C, 8 h of photoperiod per day and a relative humidity of 86%. Three weeks after, individual plants were exposed to 0.05 (control), 0.5, 1, 1.5 and 2 mM of zinc, in the form of ZnSO₄, added or not with two concentrations (9 or 15 ch: Hahnemannian centesimal) of Zincum Metallicum (ZM) for 7 days prior to the final harvest. At the harvest, fresh weight of roots and leaves were separately recorded for each treatment and used for physiological analysis. Roots and leaves were also harvested from three plants of each treatment and frozen at −80 °C or air dried for the biochemical analysis.

#### Plant growth parameters and water content

The weight of leaf and root samples were measured before (fresh weight: FW) and after (dry weight: DW) dryness at 60 °C for 3 days. Water content (WC) was determined as.

\[ WC = \frac{(FW − DW)}{FW} \times 100 \]

#### Tissue zinc content

Zinc was extracted from 25 mg dried material transferred to 125-mL conical digestion flasks. Twelve (12) milliliters of triacid mixture of nitric acid, sulfuric acid and perchloric acid (9:2:1 (v/v)) was added to the flasks. Plant materials were digested in cold for 3 h followed by digestion for 2–3 h on a hot plate, until the digest was clear or colorless. The flasks were allowed to cool, and the contents were diluted to an appropriate volume (Sahrawat et al 2002) then assayed by atomic absorption spectrophotometer (Perkin Elmer Analyst 300), using standard with known concentrations.

#### Chlorophyll and carotenoid content

Chlorophyll (Chl) and carotenoids were extracted from 5 mg of fresh leaf tissue using 25 mM Tris–HCL (pH 7.6), 1 mM EDTA, 1 mM MgCl₂ and 14 mM β-mercaptoethanol. Fifty
microliters of the extract was taken and then homogenized in 1450 μL of 80% acetone. The mixture is stored at 4 °C overnight. The samples were centrifuged for 15 min at 1500 g, and the supernatant was used to identify absorbance of chlorophyll a and b, and carotenoids at 645, 663 and 440.5 nm, respectively (Marker et al. 1980). Total chlorophyll and carotenoid content (mg g⁻¹ FW) were calculated according to the following formula:

\[
\text{Chl } a = (12.7 \times \text{DO}663) - (2.69 \times \text{DO}645) \times \frac{v}{(w \times 1000)}.
\]

\[
\text{Chl } b = (22.9 \times \text{DO}645) - (4.68 \times \text{DO}663) \times \frac{v}{(w \times 1000)}.
\]

\[
\text{Chl tot} = (20.2 \times \text{DO}645) - (8.02 \times \text{DO}633) \times \frac{v}{(w \times 1000)}.
\]

\[
\text{Carotenoids} = 46.95 \times (\text{DO}440.5 - 0.268 \times \text{Chl } a + b).
\]

Malondialdehyde determination

Oxidative damage in L. sativum was estimated based on malondialdehyde (MDA) content, which was determined from fresh leaf and root tissue, following the method of Draper and Hadley (1990). One hundred milligrams was ground in 1 mL of 0.1% trichloroacetic acid at 4 °C. After centrifugation at 15,000 g for 15 min, a 250 μL aliquot of supernatant was added to 1 mL thiobarbituric acid (prepared in 20% trichloroacetic acid) and heated for 30 min in a water bath at 95 °C. Samples were again centrifuged at 10,000 g for 10 min, and the absorbance of the supernatant was measured at 532 nm. After subtracting the non-specific absorbance at 600 nm, MDA concentration (mol g⁻¹ FW) was determined using a molar extinction coefficient of 155 mM⁻¹ cm⁻¹.

Proline content

Proline content was measured by the method of Bates et al. (1973). Fresh leaves and roots (25 mg) were mixed with 1 mL of sulfosalicylic acid (3%). The samples were centrifuged at 12,000 g for 20 min. A 500 μL aliquot of supernatant was added into 500 μL of sulfosalicylic acid, 1 mL of concentrated acetic acid and 1 mL of ninhydrin. After incubation at 100 °C for 1 h, the reaction was stopped by placing the test tube in an ice bath. Two milliliters of toluene was added to the solutions. The organic toluene phase was separated and used to determine the content of proline at 520 nm.

Protein and antioxidant enzyme assays

Fresh plant material (200 mg) was extracted in a buffer containing potassium hydrogen phosphate, EDTA, PVP and glycerol. The homogenate of each sample was centrifuged at 12,000 rpm for 10 min. The supernatant fraction was then assayed for proteins and various antioxidant enzymes.

Protein contents were analyzed according to the method of Bradford (1976), using the principle of Coomassie G250 blue binding with proteins. The concentration of protein was determined from a standard range of BSA (Bovine Serum Albumin) between 0 and 10 μg/mL⁻¹.

The superoxide dismutase (SOD) activity was determined by using the tetrathiomolybdenum blue (NTB)/riboflavin according to the method of Beuchamp and Fridovich (1971). Briefly, the sample mixture contains phosphate buffer 50 mM, (pH 7.8), EDTA 0.1 mM, L-methionine 13 mM, riboflavin 2 μM and NBT 75 μM. The reaction was initiated by exposing the reaction mixture for 15 min to a 50 μmol m⁻² s⁻¹ fluorescent light source. The absorbance was spectrophotometry measured at 560 nm.

The catalase (CAT) activity was determined spectometrically according to the method of Chaparro-Giraldo et al. (2000), by measuring the disappearance rate of hydrogen peroxide (H₂O₂) at 240 nm. The reaction mixture contained potassium phosphate buffer (50 mM, pH7), 100 μL of enzyme extract and H₂O₂ at 30%.

The glutathione peroxidase (GPx) converts H₂O₂ into water (H₂O) by the transformation of glutathione (GSH) to glutathione disulfide (GSSG). The activity of GPx was assayed by measuring the disappearance rate of H₂O₂ according to the method of Flohé and Günzler (1984). This activity was expressed in μmol of GSH/min/mg of protein.

The glutathione reductase (GR) activity was determined by monitoring the oxidation rate of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm (Rao et al. 1996). The reaction mixture (1 mL) contained 100 mM phosphate buffer (pH 7.8), 2 mM EDTA, 0.5 mM oxidized glutathione, 0.5 mM NADPH, 0.2 mM NADPH, and the reaction was initiated by adding 100 μL of the enzyme extract.

Extraction and determination of total polyphenol compounds

Roots and leaves were air-dried at room temperature for 2 weeks. Sample extracts were obtained by magnetic stirring of 1 g of dry powder per sample in methanol 80% (10 mL) for 30 min and kept at 4 °C for 24 h. The methanolic extracts obtained were filtered through a Whatman filter paper (N°0.4) and stored at 4 °C. Colorimetric quantification of the total phenolic compound was conducted using the Folin-Ciocalteu reagent, as described by Dewanto et al. (2002). Briefly, an aliquot of 125 μL of 1/10 diluted sample from each methanolic extract was dissolved in 500 μL distilled water and 125 μL Folin–Ciocalteu reagent. After shaking and resting the mixture for 3 min, 1250 μL of 7% Na₂CO₃ was added to the mixture and adjusted with distilled water to a final volume of 3 mL. The mixture was then incubated for 90 min at room temperature in the dark. The absorbance was read at 760 nm, and the total phenolic compound was
expressed as milligram gallic acid equivalent per gram of dry weight (mg GAE g⁻¹ DW) through the calibration curve of gallic acid (0–500 mg L⁻¹).

**Determination of total flavonoid content**

The total flavonoid content was determined by using a colorimetric method described previously (Zhishen et al. 1999). An aliquot (75 µL) of 7% sodium nitrite (NaNO₂) solution was added to each extract (250 µL). The mixture was shaken for 6 min before adding 0.15 µL of 10% aluminum chloride (AlCl₃). After 5 min, 0.15 mL of 1 M sodium hydroxide (NaOH) was added. The final volume was adjusted to 2.5 mL with distilled water and thoroughly mixed. Absorbance of the mixture was determined at 510 nm. Total flavonoid was expressed as milligram catechin equivalent per gram dry weight (mg CE g⁻¹ DW) using a calibration curve developed for catechin (0–500 mg L⁻¹).

**Statistical analysis**

Data were subjected to a one-way analysis of variance (ANOVA) test using ANOVA, CoStat software, version 6.4, CohortSoftware, Monterey, CA, and means were compared according to Duncan’s multiple-range test at 5% level of significance.

**Results**

**Plant growth and water content**

The effect of Zn stress on the morphology of *L. sativum* plants was marked by a decrease of shoot length in plants subjected to all Zn concentrations (0.5, 1, 1.5 and 2 mM) for 7 days (Fig. 1). Leaf and root biomass were decreased with increasing Zn concentration in the medium culture. In the leaves, these decreases reached 80% and 72% respectively under 1.5 and 2 mM Zn₂⁺. The same variation was recorded for the roots (Table 1). Zinc treatment significantly reduced tissue hydration of leaves and roots. This effect was more pronounced in the shoots since the water content dropped from 92 to 84% upon treatment with 2000 µM of Zn (Table 1).

The addition of ZM to Zn-treated plants alleviated significantly the Zn-induced growth reduction in *Lepidium sativum* L. Hence, plant subjected concomitantly to Zn and ZM produced significantly more fresh leaves as compared to their respective ones subjected to Zn alone (Table 1). This effect was more pronounced with the ZN 15 ch as compared to ZM 9 ch. For example, in plant subjected to 1.5 mM Zn, addition of ZM 15 ch increased 3 times the leaf biomass (Table 1). For the WC, the addition of ZM to the Zn-containing medium does not affect this parameter in both organs (Table 1).

**Zinc accumulation**

The concentration of Zn in the shoots and the roots of *L. sativum* plants increased with increasing the concentration of this metal in the nutrient solution (Fig. 2). For all Zn concentrations, roots accumulated more Zn than the shoots. Hence, Zn content reached 60,000 µg Zn/g DW in roots of plants exposed to 2000 µM Zn, but that of the leaves does not exceed 40,000 µg/g DW, for the same Zn dose. The addition of ZM to the nutrient solution decreased significantly Zn accumulation.
in leaves and roots compared with Zn treatment alone. For instance, Zn concentrations in leaves and roots of ZM + Zn treated plants were reduced by 20 and 11% respectively, compared to Zn-treated plants (Fig. 2). We showed also that, in the leaves, ZM 15 ch reduced more intensively the Zn accumulation than ZM 9 ch, but in the roots both ZM concentrations induced the same effect.

Table 1 Leaf and root fresh weight (g/plant) and water content (% FW) in Lepidium sativum L. grown in 0, 500, 1000, 1500 and 2000 µM zinc alone or in combination with Zincum Metallicum (9 or 15 ch) over 7 days. Means of six replicates ± standard error. For the same column, values followed by different letters are significantly different according to Duncan’s multiple-range test at 5%.

| Treatments                      | Fresh weight (g/plant) | Water content (% FW) |
|---------------------------------|------------------------|---------------------|
|                                 | Leaves | Roots | Leaves | Roots |
| Control (0 µM Zn)               | 2.61 ± 0.25a | 0.89 ± 0.05b | 92.80 ± 0.63a | 95.09 ± 0.98ab |
| 9 ch                            | 2.08 ± 0.55b | 0.59 ± 0.07a | 92.09 ± 1.08ab | 95.85 ± 0.74a |
| 15 ch                           | 1.95 ± 0.44bc | 0.70 ± 0.18ab | 92.63 ± 0.18ab | 94.67 ± 0.56ab |
| 500 µM Zn                       | 1.21 ± 0.20b | 0.49 ± 0.19b | 91.47 ± 0.49ab | 94.55 ± 1.79abc |
| 500 µM Zn + 9 ch                | 1.17 ± 0.23b | 0.50 ± 0.07ab | 90.21 ± 3.19abc | 94.56 ± 3.24abc |
| 500 µM Zn + 15 ch               | 1.81 ± 0.17ef | 0.55 ± 0.05ab | 87.04 ± 0.56de | 93.15 ± 0.35bc |
| 1000 µM Zn                      | 0.77 ± 0.40cde | 0.23 ± 0.08cd | 89.97 ± 2.75bc | 93.63 ± 1.70abc |
| 1000 µM Zn + 9 ch               | 1.08 ± 0.30ef | 0.26 ± 0.10cd | 87.72 ± 1.04cd | 92.76 ± 1.83bc |
| 1000 µM Zn + 15 ch              | 1.48 ± 0.15fg | 0.25 ± 0.07c | 84.83 ± 1.63ef | 92.76 ± 0.63cd |
| 1500 µM Zn                      | 0.51 ± 0.44de | 0.22 ± 0.04cd | 86.00 ± 0.35def | 92.63 ± 0.94bcd |
| 1500 µM Zn + 9 ch               | 0.75 ± 0.11fg | 0.19 ± 0.14cd | 86.20 ± 1.05def | 92.71 ± 0.50bcd |
| 1500 µM Zn + 15 ch              | 1.40 ± 0.20g | 0.24 ± 0.10 | 83.93 ± 2.67f | 91.96 ± 1.15 cd |
| 2000 µM Zn                      | 0.73 ± 0.27ef | 0.27 ± 0.05c | 85.91 ± 1.58def | 92.04 ± 2.47 cd |
| 2000 µM Zn + 9 ch               | 0.88 ± 0.40fg | 0.09 ± 0.05cd | 84.93 ± 2.48def | 92.53 ± 0.93bcd |
| 2000 µM Zn + 15 ch              | 1.05 ± 0.12fg | 0.25 ± 0.03d | 84.66 ± 0.56ef | 90.22 ± 1.27d |

Fig. 2 Zinc content of Lepidium sativum L. grown in 0, 500, 1000, 1500 and 2000 µM Zn alone or in combination with Zincum Metallicum (9 or 15 ch) over 7 days (a: leaf; b: root). Means of six replicates ± standard error. Values followed by different letters are significantly different according to Duncan’s multiple-range test at 5%

Chlorophyll and carotenoid content

Data illustrated in Table 2 showed that 1000, 1500 and 2000 µM Zn significantly reduced the content of total chlorophyll. In particular, the application of 2000 µM Zn caused up to 80% reduction of this pigment concentration in the leaves. On the other hand, the concentrations 1500 and 2000 µM Zn resulted in a marked reduction of carotenoids, while 500µM Zn.
and 1000 µM Zn enhanced the content of these pigments (Table 2). Zincum Metallicum at doses of 9 or 15 ch supplied to the culture medium led to a significant increase in the level of these pigments, relative to plants treated only with Zn alone.

**MDA content**

The increase in the Zn concentration in the medium induced increasing MDA content in the leaves and roots of *L. sativum* plants. As compared to control plants, the highest dose of Zn (2000 µM) increased MDA levels up to 6 and 3.5 times in leaves and roots, respectively (Fig. 3). In the presence of µM of Zn, addition of ZM increased the MDA in the shoots and roots. The supply of ZM to Zn-stressed plants reduced leaves and roots MDA content, compared to those treated only with Zn. For both organs (root and shoot), the dose 15 ch of ZM induced more important MDA reduction compared to that increased by the dose 9 ch. (Fig. 3).

**Proline accumulation**

The free proline concentration in tissues was positively correlated with the Zn concentration in the medium (Fig. 4). For instance, this amino acid concentration was increased by 7 and 5 times in leaves and roots of plant treated with 2000 µM Zn, respectively, compared to that of the control (0.05 µM Zn). The addition of ZM, at the doses 9 or 15 ch,

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### Table 2

Chlorophyll and carotenoid contents (mg g⁻¹ FW) in leaves of *Lepidium sativum* L grown in the presence of 0, 500, 1000, 1500 and 2000 µM Zn alone or in combination with Zincum Metallicum (9 or 15 ch) over 7 days. Means of six replicates ± standard error. For the same column, values followed by different letters are significantly different according to Duncan’s multiple-range test at 5%

| Treatments          | Total chlorophyll (mg g⁻¹ FW) | Carotenoids (mg g⁻¹ FW) |
|---------------------|-------------------------------|--------------------------|
| Control (0 µM Zn)   | 1.52 ± 0.12 cd                | 1.52 ± 0.01 f            |
| 9 ch                | 1.90 ± 0.08 b                 | 1.70 ± 0.01 bde          |
| 15 ch               | 2.10 ± 0.03 a                 | 1.87 ± 0.02 c            |
| 500 µM Zn           | 1.44 ± 0.03 cd                | 1.86 ± 0.01 e            |
| 500 µM Zn + 9 ch    | 1.54 ± 0.06 c                 | 1.84 ± 0.01 cd           |
| 500 µM Zn + 15 ch   | 1.90 ± 0.02 b                 | 1.94 ± 0.02 c            |
| 1000 µM Zn          | 0.92 ± 0.01 f                 | 2.40 ± 0.02 b            |
| 1000 µM Zn + 9 ch   | 1.17 ± 0.01 e                 | 2.86 ± 0.04 b            |
| 1000 µM Zn + 15 ch  | 1.40 ± 0.03 g                 | 2.96 ± 0.00 a            |
| 1500 µM Zn          | 0.64 ± 0.02 g                 | 0.90 ± 0.01 b            |
| 1500 µM Zn + 9 ch   | 0.91 ± 0.00 f                 | 1.45 ± 0.10 f            |
| 1500 µM Zn + 15 ch  | 1.15 ± 0.05 c                 | 1.56 ± 0.03 f            |
| 2000 µM Zn          | 0.33 ± 0.01 b                 | 0.72 ± 0.03 g            |
| 2000 µM Zn + 9 ch   | 0.68 ± 0.02 e                 | 0.93 ± 0.00 h            |
| 2000 µM Zn + 15 ch  | 0.88 ± 0.02 f                 | 1.10 ± 0.01 g            |

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Fig. 3 MDA content of *Lepidium sativum* L grown in 0, 500, 1000, 1500 and 2000 µM Zn alone or in combination with Zincum Metallicum (9 or 15 ch) over 7 days (a: leaf; b: root). Means of six replicates ± standard error. Values followed by different letters are significantly different according to Duncan’s multiple-range test at 5%
further enhanced the content of proline, to reach up to 56 and 31\% in leaves and roots of plants treated with 2000 \( \mu \text{M} \) Zn, respectively (Fig. 4).

Under control condition of Zn, the addition of ZM enhanced the accumulation of proline in the leaves and in the roots.

**Enzyme activities**

Data presented in Figs. 5 and 6 showed that the activities of SOD, CAT, GPX and GR varied to a different extent depending on Zn and ZM concentrations and the plant organ. As compared to that in control plants (0.05 \( \mu \text{M} \) Zn), the activity of SOD in the leaves was not significantly altered in Zn-treated plants, whereas it was significantly increased in roots of Zn-treated plants with 500, 1000 and 1500 and it was dropped in the roots of at 2000 \( \mu \text{M} \) Zn-treated plants (Fig. 5a, b). In plant cultivated under 0.05 \( \mu \text{M} \) Zn, the addition of ZM induced light and not significant increase in SOD activity in leaves and significant increase only at 15 ch.

CAT activity was significantly increased in both leaves and roots of plant treated with 500, 1000 and 1500, except that in roots of 500 \( \mu \text{M} \) Zn treated. Nevertheless, the activity of this enzyme was dropped by 20\% and 66\% in both leaves and roots of plants treated with 2000 \( \mu \text{M} \) Zn, respectively (Fig. 5c, d). GPX and GR activities were increased or maintained stable across all the Zn treatments (Fig. 5a, b). At equal Zn concentrations, the leaves and roots of ZM-Zn treated plants showed highest activities of the above-mentioned enzymes than those of the Zn alone-treated plants.

**Accumulation of phenolic compounds**

Similarly to the antioxidant enzyme activities, the total polyphenol and flavonoid contents varied to a different extent depending on Zn concentration and treatment as well as plant organ. The total polyphenol content was not significantly altered in leaves and roots of plants treated with Zn alone for all used doses, except that of roots treated with 1500 and 2000 \( \mu \text{M} \) (Table 3). The flavonoid content significantly increased in leaves and roots of plants treated with 1000, 1500 and 2000 \( \mu \text{M} \) Zn (Table 3). Under adequate zinc concentration in the medium (0.05 \( \mu \text{M} \)), the addition of ZM, at 9 and 15 ch, did not induce significant changes in total polyphenols and flavonoids in the roots and the leaves of *L. sativum* plants. The combined treatment of Zn with ZM increased the total polyphenol and flavonoid levels in both organs compared to that treated with Zn alone, mainly in the presence of 2000 \( \mu \text{M} \) and the ZM dose at 15 ch (Table 3).

**Discussion**

Garden cress (*Lepidium sativum* L.) is one of the edible medicinal plants in Tunisia, providing an important source of proteins, carbohydrates, dietary fibers and minerals including calcium, phosphorus, potassium, zinc (Alqahtani et al. 2019; Manohar et al. 2012).

In the current study, we focused on improving zinc tolerance of this species to increase its agricultural production. The study evaluated, for the first time, the capability of the homeopathic drug Zincum Metallicum (ZM) to promote the
overall performance and productivity of *L. sativum* under Zn stress. Indeed, the results showed that fresh weight of *L. sativum* plants was significantly reduced after exposure to 1000 µM Zn along with the appearance of necrotic symptoms in leaves. This decrease was markedly pronounced in plants grown under 2000 µM Zn. Our results strongly suggest the sensitivity of *L. sativum* to Zn excess. Hence, under Zn treatment, water contents were significantly reduced, mainly in leaves, suggesting that Zn induced also tissue dehydration in this species. In a previous study, the fresh weight of *Pisum sativum* L. was significantly reduced at different Zn concentrations (35, 70, 350, 700 µM) (Stoyanova and Doncheva 2002), showing signs of toxicity essentially leaf chlorosis. In the study of Kastori et al. (2008), the relative water content was significantly decreased in sunflower plants subjected to Zn excess.

According to our study, the uptake of Zn increased in leaves and roots with increasing Zn concentration in the medium. We showed also that Zn accumulation was more than 98% in roots compared to leaves. These results suggest that *L. sativum* adopts root accumulation of Zn\(^{2+}\) as solution to prevent its transport to the photosynthetic organs.

Reductions in plant growth due to heavy metal accumulation are often associated with enhanced pigment degradation. In *L. sativum*, a significant decline in its total chlorophyll levels was noticed in plants subjected to 1000, 1500 and 2000 µM Zn concentrations. Similar results were reported by Samreen et al. (2017) in different varieties of mung beans treated with 2 µM of Zn. The Zn toxicity may be induced by either the decrease in chlorophyll synthesis or the increase in its degradation or to the inhibition of photosynthetic electron transport (Vaillant et al. 2005). Carotenoid content was markedly increased under exposure to 500 and 1000 µM Zn, suggesting that this metabolite was less sensitive to the Zn treatment. Under mercury exposure, Smolinska et al. (2017) also reported that carotenoid content increased in leaves of *L. sativum* plants.

![Fig. 5](image.png)

Superoxide dismutase and catalase activities in leaves (a and c respectively) and roots (b and d respectively) of *Lepidium sativum* L. grown in 0, 500, 1000, 1500 and 2000 µM zinc alone or in combination with Zincum Metallicum (9 or 15 ch) over 7 days. Means of six replicates ± standard error. Values followed by different letters are significantly different according to Duncan’s multiple-range test at 5%
The exposure of plants to heavy metals resulted often from the inevitable oxidative stress, considered as the main factor responsible for membrane cell damage (Smeets et al. 2008). It has been demonstrated that oxidative stress in *L. sativum* was induced by different trace elements including mercury (Smolinska et al. 2017), copper (Rombel-Bryzek et al. 2017) and arsenic (Umar et al. 2013) and other abiotic stressors like salinity, drought, temperature and light exposure (Al-Sammarraie et al. 2020). Under such constraints, the effects of oxidative stress on membrane peroxidation are often estimated by determining the content of MDA, which is used as an indicator of membrane lipid peroxidation. The results obtained in this study revealed that elevated zinc concentrations (1500 and 2000 µM) increased MDA content in the leaves and roots of *L. sativum* plants. This effect can be explained by the overproduction of ROS and/or the disturbance of the antioxidant system inducing hence lipid-membrane peroxidation as demonstrated by Chaoui et al. (1997). It has been demonstrated that Zn induced acute oxidative stress in *Triticum aestivum* (Khan et al. 2007). Besides, this phenomenon can be initiated by the iron-containing enzyme lipoxygenase, a membrane-bound enzyme, which is known to oxidize polyunsaturated fatty acids and to produce free radicals, mediating thus lipid peroxidation (Chaoui et al. 1997).

To cope with oxidative stress, the plant activates its antioxidant defense system, which consists of osmoticum, enzymes and antioxidant molecules. For instance, the proline known for its role as an osmoticum would act as scavenging agent of ROS including singlet oxygen (1O2).

![Fig. 6](image) Glutathione peroxidase and glutathione reductase activities in leaves (a and c respectively) and root (b and d respectively) of *Lepidium sativum* L. grown in 0, 500, 1000, 1500 and 2000 µM zinc alone or in combination with Zincum Metallicum (9 or 15 ch) over 7 days. Means of six replicates ± standard error. Values followed by different letters are significantly different according to Duncan’s multiple-range test at 5%.
The treatment of plants with zinc induces a more or less significant increase in catalase activity both in the leaves and in the roots. In a previous study, Jain et al. (2010) revealed that Zn stimulated CAT in sugar cane. Besides, the current study revealed that Zn enhanced the accumulation of polyphenols and flavonoids in the leaves and roots of L. sativum. This accumulation is more marked in the leaves than in the roots. This endogenous increase can be attributed to their potential antioxidant and their ability to eliminate ROS (Achat et al. 2016). Bartakova et al. (2020) also found that the main compounds possessing antioxidant activity in the extracts of vermelho wood assessed by UHPLC analysis were hydroxycinnamic acids.

To alleviate Zn-induced toxicity in L. sativum, the homeopathic drug, ZM, was assessed in plant treated with different Zn concentrations (500, 1000, 1500 and 2000). Results showed that ZM alleviated

and hydroxyl radical (OH\(^-\)), thus ensuring the protection of cellular structure and functioning (Sharmila and Pardha, 2002). The results of our study revealed that Zn significantly increased accumulation of proline in plants treated with zinc. In the same context, the study of Li et al. (2013) reported that constitutive osmotic regulator concentrations including proline, total soluble proteins and total soluble sugars were enhanced in the leaves of wheat plants treated with zinc.

On the other hand, our results revealed that Zn (500, 1000 and 1500 µM) stimulated the root activity of SOD, while that of leaves was unchanged across all the Zn treatment. SOD stimulation is also observed in many plants subjected to Zn-metallic constraint, such as beans (Michael and Krishnaswamy 2011). Catalase is an enzyme that catalyses the decomposition of H\(_2\)O\(_2\) into oxygen and H\(_2\)O. The treatment of plants with zinc induces a more or less significant increase in catalase activity both in the leaves and in the roots. In a previous study, Jain et al. (2010) revealed that Zn stimulated CAT in sugar cane. However, in this study we revealed that CAT activity was inhibited by 2000 µM Zn, suggesting the incapacity of L. sativum to tolerate high concentration of Zn. In addition, the activity of GPX and GR activities was increased or maintained stable across all the Zn treatments. Glutathione peroxidase (GPX) was stimulated or maintained unchanged across all the Zn treatment. These results confirm those obtained in the study carried out by Duman and Ozturk (2010) on

| Treatments          | Total polyphenol content                  | Total flavonoid content                  |
|---------------------|------------------------------------------|-----------------------------------------|
|                     | (mg EGA/g Ms)                             | (mg EGA/g Ms)                           |
|                     | Leaves | Roots | Leaves | Roots |
| Control (0 µM Zn)   | 1.42 ± 0.05c | 0.40 ± 0.05 b | 3.52 ± 0.36f | 1.74 ± 0.41 f |
| 9 ch                | 1.53 ± 0.34de | 0.56 ± 0.30ab | 4.01 ± 0.14ab | 1.92 ± 0.05f  |
| 15 ch               | 1.60 ± 0.04cde | 0.33 ± 0.04 b | 4.74 ± 0.32ab | 2.05 ± 1.06f  |
| 500 µM Zn           | 1.44 ± 0.14cde | 0.63 ± 0.05gh | 4.61 ± 0.10gh | 1.84 ± 0.02f  |
| 500 µM Zn + 9 ch    | 1.67 ± 0.06cde | 0.58 ± 0.09gh | 5.12 ± 0.13gh | 2.47 ± 0.16f  |
| 500 µM Zn + 15 ch   | 1.82 ± 0.14cde | 1.40 ± 0.05g  | 5.80 ± 0.18f  | 2.67 ± 0.02f  |
| 1000 µM Zn          | 1.53 ± 0.15cde | 0.70 ± 0.03fgh | 5.34 ± 0.09gh | 2.39 ± 0.13f  |
| 1000 µM Zn + 9 ch   | 1.97 ± 0.56hde | 1.01 ± 0.07bc  | 6.35 ± 0.33df | 3.41 ± 0.13f  |
| 1000 µM Zn + 15 ch  | 2.13 ± 0.55bd | 1.79 ± 0.01b  | 7.07 ± 0.36f  | 3.80 ± 0.00cd |
| 1500 µM Zn          | 1.72 ± 0.18cde | 0.79 ± 0.02efg | 7.22 ± 0.39f  | 2.48 ± 0.16f  |
| 1500 µM Zn + 9 ch   | 2.24 ± 0.48bc | 1.21 ± 0.04cd | 8.78 ± 0.33cd | 3.67 ± 0.05cd |
| 1500 µM Zn + 15 ch  | 2.89 ± 0.59a  | 2.39 ± 0.11a  | 9.67 ± 0.04bc | 4.16 ± 0.01bc |
| 2000 µM Zn          | 1.72 ± 0.09cde | 0.96 ± 0.12def | 8.47 ± 0.11d  | 3.37 ± 0.18d  |
| 2000 µM Zn + 9 ch   | 2.55 ± 0.04ab | 1.28 ± 0.04sd | 10.17 ± 0.09ab | 4.54 ± 0.14b  |
| 2000 µM Zn + 15 ch  | 2.89 ± 0.07a  | 2.43 ± 0.16a  | 10.87 ± 0.22a | 4.54 ± 0.02a  |

Table 3 Polyphenol and flavonoid content of Lepidium sativum L grown in 0, 500, 1000, 1500 and 2000 µM zinc alone or in combination with Zinicum Metallicum (9 or 15 ch) over 7 days. Means of six replicates ± standard error. For the same column, values followed by different letters are significantly different according to Duncan’s multiple-range test at 5%.
significantly Zn-induced phyto-toxic effect. The improvement concerned the growth parameters and was manifested by increases in the fresh biomass and in the content of photosynthetic pigments concomitant to a reduction in the accumulation of Zn$^{2+}$ especially in the leaves, in plants treated with the combination of zinc and ZM compared to the plant treated only by zinc. The antioxidant enzyme activity, superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and glutathione reductase (GR), is increased in the presence of zinc. However, this stimulation was more important in the presence of homeopathy. In addition to the antioxidative enzymes, other molecules could play important roles in the alleviation of zinc toxicity in *Lepidium sativum* L. as proline, polyphenols and flavonoids. In fact, the biosynthesis of these compounds was stimulated by Zn, and this overproduction was more important under combined treatment zinc-homeopathy drug. Thus, in this study, we were able to demonstrate that the addition of ZM in a culture medium can significantly alleviate zinc toxicity by activating the antioxidant system and the production of antioxidative molecules. The activation of the antioxidant defense seems to be the reason allowing plants to cope with zinc toxicity. Similar results were reported by Mazón-Suástegui et al. (2019), showing that the homeopathy is able to stimulate favorable biological and even genetic responses in *Ocimum basilicum* L., *Phaseolus vulgaris* L., *Cucumis sativus* L. and *Solanum lycopersicum* L. growing under different abiotic constraints. In addition, Banerjee and Sukul (2013) reported that homeopathic compounds might reduce the toxic effect of copper by promoting seed germination and peroxidase activity in *Vigna unguiculata* L.

To the best of our knowledge, no more studies were conducted using homeopathy to elevate heavy metal toxicity, but Mazón-Suástegui et al. (2020) found that the treatment of the common bean, *P. vulgaris* with Natrum muriaticum homeopathic drug, was able to alleviate the salt (NaCl) stress by increasing the morphometric variables and photosynthetic rate.

Other substances were used to reduce zinc toxicity like hydrogen sulfide especially in roots of *Solanum nigrum* L. plants (Liu et al. 2015). Salicylic acid and nitric oxide were also used in *Carthamus tinctorius* L plants and improved the activity of ascorbate–glutathione cycle enzymes, and those enzymes are involved in glyoxalase system as compared to the plants treated with Zn only (Namdjovan et al. 2017).

Overall, the results obtained in this study suggest that the use of ZM has a great potential in sustainable organic agriculture, and its application can contribute to increasing the organic productivity of *Lepidium sativum* L.

### Conclusion

In summary, our findings showed high sensitivity of *Lepidium sativum* L. to zinc stress as indicated by the reduced growth of leaf and root, enhanced zinc ion accumulation, degradation of photosynthetic pigments and amplified level of malondialdehyde (MDA). The increase of the antioxidative enzymes activities and content of proline, total polyphenols and flavonoids, by Zn stress, was however unable to restore the morpho-physiological parameters, to inhibit lipid peroxidation and restrict the accumulation of Zn$^{2+}$ in leaves and roots. The ZM supply with Zn treatment reduced the toxicity symptoms of this metal to some extent. In fact, the ameliorative effect of this homeopathic drug on plant biomass production was paralleled by the restoration of the pigment amounts and the inhibition of MDA content along with higher production of proline and polyphenols and an improvement of the antioxidant enzyme activities (SOD, CAT, GPx and GR). Our obtained results indicate that the presence of ZM mainly at the dose 15 ch affects different physiological and biochemical aspects in *Lepidium sativum* L. plants in response to zinc stress, and this could be recognized as an adaptive mechanism against heavy metal constraints.

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### Author contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by GB. Conceptualization was made by CCH. The first draft of the manuscript was written by GB, SBA and TG and all authors commented on previous versions of the manuscript. Formal analysis was made by AC. All authors read and approved the final manuscript.

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### Data availability

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

### Declarations

**Ethics approval and consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.
References

Abo El Maati M, Labib S, Al Gaby A, Ramadan M (2016) Antioxidant and antibacterial properties of various extracts of garden cress (Lepidium sativum L.). Zagazig J Agric Res 43:1685–1697. https://doi.org/10.21608/zjar.2016.98127

Achat S, Rakotomanonana N, Madani K, Dangles O (2016) Antioxidant activity of olive phenols and other dietary phenols in model gastric conditions: scavenging of the free radical DPPH and inhibition of the haem-induced peroxidation of linoleic acid. Food Chem 213:135–142. https://doi.org/10.1016/j.foodchem.2016.06.076

Alqahtani FY, Aleainzy FS, Mahmood AZ, Farshori NN, Alfaraj R, Alsheddi ES, Alsarra IA (2019) Chemical composition and antimicrobial, antioxidant, and anti-inflammatory activities of Lepidium sativum seed oil. Saudi J Biol Sci 26:1089–1092. https://doi.org/10.1016/j.sjbs.2018.05.007

Al-Sammarraie ON, Alsharafa KY, Al-lmoun MO, Khleifat KM, Al-Sarayreh SA, Al-Shuneigat JM, Kalaji HM (2020) Effect of various abiotic stressors on some biochemical indices of Lepidium sativum plants. Sci Rep 10:21131. https://doi.org/10.1038/s41598-020-78303-1

Arnon DI, Hoagland DR (1940) Crop production in artificial culture solutions and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. Soil Sci 50:463–485

Banerjee P, Sukul S (2013) Cuprum Sulphuricum—a homeopathic dant and antibacterial properties of different extracts of garden cress (Lepidium sativum L.). Zagazig J Agric Res 43:1685–1697. https://doi.org/10.21608/zjar.2016.98127

Bartakova M, Dvorackova E, Chromcova L, Hrdlicka P (2020) Induction of oxidative stress and antioxidant responses in Vigna mungo by zinc stress. Russ J Plant Physiol 58:85–91. https://doi.org/10.1134/s1021443711010079

Banerjee P, Sukul S (2013) Cuprum Sulphuricum—a homeopathic dant and antibacterial properties of different extracts of garden cress (Lepidium sativum L.). Zagazig J Agric Res 43:1685–1697. https://doi.org/10.21608/zjar.2016.98127

Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assay and assay applicable to acrylamide gels. Anal Biochem 44:276–287. https://doi.org/10.1016/0003-2697(71)90370-8

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0021-9355(76)90527-3

Braha B, Tintemann H, Krauss G, Bärlocher F, Krauss G-J (2016) Alteration in Cd-induced gene expression under nitrogen deficiency in Hordeum vulgare. Plant Cell Environ 26(6):821–833. https://doi.org/10.1111/pce.12101

Doillon D (2010) Molecular determinants of zinc tolerance in eukaryotic microorganisms. Dissertation Henri Poincaré University, Forest Biology. 223p. https://hal.univ-lorraine.fr/tel-

Dewanto V, Wu X, Adom KK, Liu RH (2002) Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agric Food Chem 50(10):2427–2434. https://doi.org/10.1021/jf0115589

Draper H, Hadley M (1990) Malondialdehyde determination as index of lipid peroxidation. Oxygen Radicals in Biological Systems Part B: Oxygen Radicals and Antioxidants 421–431.https://doi.org/10.1016/0076-6789(90)86135-I

Duman F, Ozurtuk F (2010) Nickel accumulation and its effect on biomass, protein content and antioxidative enzymes in roots and leaves of watercress (Nasturtium officinale R.Br.). J Environ Sci 22(4):526–532. https://doi.org/10.1016/j.ijjes.2009.06137-6

Draper H, Hadley M (1990) Malondialdehyde determination as index of lipid peroxidation. Oxygen Radicals in Biological Systems Part B: Oxygen Radicals and Antioxidants 421–431. https://doi.org/10.1016/0076-6789(90)86135-I

Duman F, Ozurtuk F (2010) Nickel accumulation and its effect on biomass, protein content and antioxidative enzymes in roots and leaves of watercress (Nasturtium officinale R.Br.). J Environ Sci 22(4):526–532. https://doi.org/10.1016/j.ijjes.2009.06137-6

Feng-tao LI, Jian-min QI, Gao-yang Z, Li-huil, Ping-ping F, Ai fen T, Jian-Tan XU (2013) Effect of cadmium stress on the growth, antioxidative enzymes and lipid peroxidation in two kenaf (Hibiscus cannabinus L.) Plant Seedlings. J Integr Agric 12(4):610–620. https://doi.org/10.1007/s00195-013-6027-9

Finkemeyer I, Kluge I, Metwally A, Georgi M, Grotjohann N, Dietz K (2003) Alteration in Cd-induced gene expression under nitrogen deficiency in Hordeum vulgare. Plant Cell Environ 26(6):821–833. https://doi.org/10.1111/pce.12101

Flohé L, Günzler WA (1984) Assays of glutathione peroxidase. Methods in Enzymology 114–120. https://doi.org/10.1016/S0076-6879(84)80515-1

Gong B, He E, Qiu H, Van Gestel CAM, Romero-Freire A, Zhao L, Xu X, Cao X (2020) Interactions of arsenic, copper, and zinc in soil-plant system: partition, uptake and phytotoxicity. Sci Total Environ 745:140926. https://doi.org/10.1016/j.scitotenv.2020.140926

Greger B, Pathak GC, Pandey N (2011) Induction of oxidative stress and antioxidant responses in Vigna mungo by zinc stress. Russ J Plant Physiol 58:85–91.https://doi.org/10.1134/s1021443711010079

Haleng J, Pincermall J, Defraigne-JO, Charlier C, Chapelle J-P (2007) Le stress oxidant. Revue Médicale de Liége 62(10):628–638. http://hdl.handle.net/2268/8914

Jain R, Srivastava S, Solomon S, Shrivastava AK, Chandra A (2010) Impact of excess zinc on growth parameters, cell division, nutrient accumulation, photosynthetic pigments and oxidative stress of sugarcane (Saccharum spp.). Acta Physiol Plant 32:979–986. https://doi.org/10.1007/s11738-010-0487-9

Kastori R, Petrovic M, Petrovic N (2008) Effect of excess lead, cadmium, copper, and zinc on water relations in sunflower. J Plant Nutr 15(11):2427–2439. https://doi.org/10.1080/0190416920 9264885

Khan NA, Samuillah NS, Nazar R (2007) Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (Triticum aestivum) cultivars differing in yield potential under cadmium stress. J Agron Crop Sci 193:435–444. https://doi.org/10.1111/j.1399-6100.2007.00272.x

Li L, Huang X, Borthakur D, Ni H (2012) Photosynthetic activity and antioxidative response of seagrass Thalassia hemprichii to trace metal stress. Acta Oceanol Sin 31:98–108. https://doi.org/10.1007/s13131-012-0210-3

Li X, Yang ANY, Jia L, Chen BH, Wei BX (2013) Zinc-induced oxidative damage, antioxidant enzyme response and proline metabolism in roots and leaves of wheat plants. Ecotoxicol Environ Saf 89:150–157. https://doi.org/10.1016/j.ecoenv.2012.11.025

Lin RZ, Wang XR, Lui Y, Wu DC, Guo HY, Yin DQ (2007) Effects of soil cadmium on growth, oxidative stress and antioxidative system in wheat seedlings (Triticum aestivum L.), Chemosphere 69:89–98. https://doi.org/10.1016/j.chemosphere.2007.04.041

Liu X, Chen J, Wang G-H, Wang W-H, Shen Z-J, Luo M-R, Gao G-F, Simon M, Ghoto K, Zheng H-L (2015) Hydrogen sulfide alleviates zinc toxicity by reducing zinc uptake and regulating genes expression of antioxidative enzymes and metallothioneins in roots of the cadmium/zinc hyperaccumulator Solanum nigrum L. Plant Soil 400:177–192. https://doi.org/10.1007/s11104-015-2719-7

J Clin Biochem Technol 22:253–264. https://doi.org/10.1007/s12892-019-0097-0

J Agric Food Chem 50(10):3010–3014. https://doi.org/10.1021/jf0115589
Manohar D, Viswanatha GL, Nagesh S, Jain V, Shivprasad HN (2012) Ethnopharmacology of *Lepidium sativum* linn (Brassicaceae): a review. Int J Phyther Res 2(1):1–7

Marker AFH, Nush EA, Rai H, Rieman NB (1980) The measurement of photosynthetic pigments in freshwaters and standardization of methods: conclusions and recommendations. Arch Hydrobiol Beih (ergelnshm): 14:91–106

Mazón Suástegui JM, Ojeda Silvera CM, García Bernal MR, Avilés-Rout GR, Das P (2003) Effect of metal toxicity on plant growth and metabolism. Arch Hydrobiol Beih (ergelnshm): 14:91–106

Mazón Suástegui JM, Ojeda Silvera CM, García Bernal MR, Avilés-Rout GR, Das P (2003) Effect of metal toxicity on plant growth and metabolism. Arch Hydrobiol Beih (ergelnshm): 14:91–106

Mazón-Suástegui JM, Ojeda-Silvera CM, García-Bernal M, Avilés-Rout GR, Das P (2003) Effect of metal toxicity on plant growth and metabolism. Arch Hydrobiol Beih (ergelnshm): 14:91–106

Mazón-Suástegui JM, Ojeda-Silvera CM, García-Bernal M, Avilés-Rout GR, Das P (2003) Effect of metal toxicity on plant growth and metabolism. Arch Hydrobiol Beih (ergelnshm): 14:91–106

Rajfur M, Zhuk O (2017) The impact of copper stress on growth rate, chlorophyll, protein and mineral contents of hydroponically grown mungbeans plant (*Vigna radiata*). Arab J Chem 10:S1802–S1807. https://doi.org/10.1016/j.arabjc.2013.07.005

Sharmila P, Pardha SP (2002) Proline accumulation in heavy metal stressed plants: an adaptive strategy. Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants 179–199. https://doi.org/10.1007/978-94-017-2660-3_7

Smeets K, Ruymink J, Semane B, van Bellemhe F, Remans T, van Sanden S, Vangronsveld J, Cuypers A (2008) Cadmium-induced transcriptional and enzymatic alterations related to oxidative stress. Environ Exp Bot 63. https://doi.org/10.1016/j.envexpbot.2007.10.028

Smolinska B, Leszczynska J (2017) Photosynthetic pigments and peroxidase activity of *Lepidium sativum* L. during assisted Hg phytoextraction. Environ Sci Pollut Res 24:13384–13393. https://doi.org/10.1007/s11356-017-8951-3

Smolinska B, Szczodrowska A, Leszczynska J (2017) Protein changes in *Lepidium sativum* L. exposed to Hg during soil phytoremediation. Int J Phytochem 19:765–773. https://doi.org/10.1080/15226158.2017.1284754

Soyanova Z, Doncheva S (2002) The effect of zinc supply and succinate treatment on plant growth and mineral uptake in pea plant. Braz J Plant Physiol 14:111–116. https://doi.org/10.1590/s1677-04202002000200005

Todeschini V, Minga G, D’Agostino G, Carniato F, Roccotiello E, Bertolino M (2017) Comparative study of responses in four Datura species to a zinc stress. Braz J Plant Physiol 14:111–116. https://doi.org/10.1590/s1677-04202002000200005

Tsonev T, Lidon F (2012) Zinc in plants—an overview. Emirates J Food Agric 24(4):322–333

Umar S, Gauba N, Anjum NA, Siddiqi TO (2013) Arsenic toxicity in garden cress (*Lepidium sativum* Linn.): significance of potassium nutrition. Environ Sci Pollut Res 20:6039–6049. https://doi.org/10.1007/s11356-013-1624-y

Vaillant N, Monnet F, Hitmi A, Sallanon H, Coudret A (2005) Comparative transcriptional and enzymatic alterations related to oxidative stress. Physiol and Biochem Plants 60:497–508. https://doi.org/10.1007/s11356-015-7542-1

Veenen K, Van Buit B, Nolte H, Tews J, van den Berg M (2017) Zinc effect on growth rate, chlorophyll, protein and mineral contents of hydroponically grown mungbeans plant (*Vigna radiata*). Arab J Chem 10:S1802–S1807. https://doi.org/10.1016/j.arabjc.2013.07.005

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