Ecotoxicology (2022) 31:92–113
https://doi.org/10.1007/s10646-021-02492-5

Metabolic alterations elicited by Cd and Zn toxicity in *Zea mays* with the association of *Claroideoglomus claroideum*

Edappayil Janeeshma[1] · Jos T. Puthur[1] · Jacek Wróbel[2] · Hazem M. Kalaji[3,4]

Accepted: 4 October 2021 / Published online: 29 October 2021

Abstract

The concentrations of cadmium (Cd) and zinc (Zn) in arable lands exceed the maximum permissible levels due to the excessive use of phosphorus fertilizers and fungicides by farmers. The increasing issues related to the application of agrochemicals have lead to the demand for the implementation of sustainable agricultural approaches. Association of arbuscular mycorrhizae with crop plants is an appropriate strategy due to the potential of these microorganisms to augment the metals tolerance of plants through the immobilization of Cd and Zn in an eco-friendly manner. In the present study, 45 d old *Zea mays* (var. CoHM6) plants inoculated with AM fungi (*Claroideoglomus claroideum)* were exposed to 1.95 g Zn Kg^{-1} soil and 0.45 g Cd Kg^{-1} soil. The major objective of this study was to determine the metabolic alterations in the leaves and roots of mycorrhizal and non-mycorrhizal plants exposed to $CdCl_2$ and $ZnSO_4$. Both non AM and AM plants exhibited alterations in the quantity of primary and secondary metabolites on exposure to Zn and Cd toxicity. Moreover, Zn and Cd-induced accumulation of γ-sitosterol reduced the quantity of neophytadiene (a well-known terpenoid) and aided the production of 3-β-acetoxystigmasta-4,6,22-triene in maize leaves. Mycorrhization and heavy metal toxicity induced significant metabolic changes in the roots by producing 4,22-stigmastadiene-3-one, eicosane, 9,19-cyclolanost-24-en-3-ol, pentacosane, oxalic acid, heptadecyl hexyl ester, l-norvaline, and n-(2-methoxyethoxycarbonyl). In addition, the metal-induced variations in leaf and root lignin composition were characterized with the aid of the FTIR technique. Mycorrhization improved the tolerance of maize plants to Cd and Zn toxicity by stabilizing these metal ions in the soil and/or limiting their uptake into the plants, thus ensuring normal metabolic functions of their roots and shoots.

Keywords Arbuscular Mycorrhiza – AM · Cadmium · GC-MS · Mycorrhiza · Phytochemistry · Zinc

Introduction

In the present scenario, heavy metal (HM) contamination in agricultural land increases the intensity of the biomagnification of toxic metal ions in edible crops and thereafter in the human body, which causes serious health issues. As in animals, plants also suffer from the negative impact of these xenobiotics by decreasing the rate of photosynthesis, reducing the yield, impairing different developmental processes, hindering the mineral acquisition, and accumulating reactive oxygen species (ROS) (Mishra and Sangwan 2019). Of the different metal contaminants, cadmium (Cd) and zinc (Zn) are highly toxic because of the high solubility of these metals. Cadmium is introduced into the environment by anthropogenic activities, and the crop plants surviving in high levels of Cd exhibits many physiological disorders (Rostami et al. 2020). At the same time, Zn is an essential element and becomes toxic only when it exceeds the tolerable level (Kaya et al. 2018).

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s10646-021-02492-5.

✉ Jos T. Puthur
jtputhur@yahoo.com

[1] Plant Physiology and Biochemistry Division, Department of Botany, University of Calicut, C.U. Campus P.O, Kerala 673635, India

[2] Department of Bioengineering, West Pomeranian University of Technology in Szczecin, 17 Słowackiego Street, 71-434 Szczecin, Poland

[3] Department of Plant Physiology, Institute of Biology, Warsaw University of Life Sciences, Nowoursynowska 159, 02-776 Warsaw, Poland

[4] Institute of Technology and Life Sciences (ITP), Falenty, Al. Hrabska 3, 05-090 Raszyn, Poland

Phytoremediation is a sustainable technique in which the metal tolerance potential of plants are utilized for the reclamation of heavy metal contaminated lands (Sarath and Puthur 2020). *Zea mays* is one of the important crop plants with metal tolerance potential, and the phytostabilization of metal ions in the rhizosphere could increase its level of tolerance (Tovar-Sánchez et al. 2018; Naz et al. 2021). Further enhancement in its metal tolerance potential is possible with the association of different soil microorganisms (Shahzad et al. 2021). Of the different micro-fauna used for this purpose, mycorrhizae can be considered as the best candidate as it is an eco-friendly and cost-effective method (Janeeshma and Puthur 2020; Adeyemi et al. 2021). The mycorrhizal association is an interesting achievement of co-evolution in the biological world, and this symbiotic association is an established strategy of stress tolerance in plants (Liu et al. 2014; Begum et al. 2019).

Metabolic alterations associated with heavy metal toxicity, especially with Cd and Zn toxicity was reported in multiple works published earlier (Garg and Singh 2018; Sruthi and Puthur 2019). Arbuscular mycorrhizal (AM) fungi have been demonstrated to alleviate heavy metal stress on plants and thus provide an attractive platform to advance plant-based environmental clean-up (Mendoza et al. 2015). AM can alter the concentration of heavy metals in plants by immobilizing heavy metals in the cell wall of intra- or extra-radicular hyphae, chelating the metals by secretion of several compounds such as glomalin, or by compartmentalization of metals in fungal cells (Janeeshma and Puthur 2020). Changes in plant metabolite concentrations of AM plants may be due to enhanced mineral nutrition and/or plant reaction to fungal colonization (Rivero et al. 2015). *Rhizophagus irregularis* and *Funneliformis mosseae* aid to improve the nutritional status with a strong metabolic rearrangement in roots of tomato (Rivero et al. 2015). Nevertheless, changes in primary and secondary metabolic composition in mycorrhizal and non-mycorrhizal plants, emphasizing the role of these metabolites in improving metal tolerance potential, have not been well studied (Garg and Singh 2018). The present study focuses on the alterations in the primary and secondary metabolic composition in mycorrhizal and non-mycorrhizal plants, emphasizing the role of these metabolites to improve the metal tolerance potential of maize plants. We hypothesize that the association of arbuscular mycorrhizal fungi will protect the maize plants by improving the tolerance against trace element toxicity by altering the metabolomics.

Material and methods

Plant material, fungal inoculum, growth conditions and treatments

Maize (variety CoHM 6) seeds were collected from Centre for Plant Breeding and Genetics, Department of Millets, Tamil Nadu Agriculture University (TNAU), Coimbatore, India and AM (*Claroideoglomus claroideum*) inoculum was obtained from the Centre for Mycorrhizal Culture Collection (CMCC), The Energy and Resources Institute (TERI), New Delhi.

Growth conditions and treatments

Surface sterilized maize seeds (3 nos) were placed 8 cm below the sterilized soil filled in polythene bags (18 × 13 cm). Two sets of plants were maintained for the experiment, one with AM inoculation and the other without AM inoculation. For AM inoculation 20 g of *C. claroideum* inoculum (containing approximately 320 spores per gram) was introduced into the soil. These polythene bags were kept in polyhouse maintained at 60 ± 2% relative humidity, 25 ± 2 °C temperature and 12 h daylight ranging from 700–900 µmol/m^2/s. Plants were initially watered with distilled water and fertilized with 50 mL of quarter-strength modified Hoagland solution (The Hoagland solution was prepared by avoiding $ZnSO_4$. $7H_2O$). After 45 d of growth, mycorrhizal and non mycorrhizal plants were treated with 40 mL (field capacity of the soil) solutions containing 1.95 g Zn Kg^{-1} soil as $ZnSO_4$ and 0.45 g Cd Kg^{-1} soil as $CdCl_2$. For the selection of these stress imparting concentrations, plants were exposed to different concentrations of $CdCl_2$ (0, 0.225, 0.45, 0.675 g Cd Kg^{-1} soil) and $ZnSO_4$ (0.0, 0.65, 1.30, 1.95 g Zn Kg^{-1} soil) and analysis of various parameters were conducted in 4 d intervals (0, 4, 8 and 12 d). The second lower leaf and the roots of the maize plants were taken for various analysis, where the non AM plants unexposed to any metal stress were considered the control. The experiments were conducted as three independent experiments with three replicate, and a randomized block design (RBD) was adopted for the experiment.

Multiplication of inoculum

For the multiplication of *C. claroideum* spores, the pot culture method was adopted. Pots were filled with 3 kg of sterilized soil (soil:sand in 1:1). Soil sterilization was done according to the method of Raj and Sharma (2009), and the physicochemical characteristics of this soil were determined as; 72.50% sand, 6.25% silt, 21.25% clay, pH 6.8, 1.26% of organic carbon, 6200 mg kg^{-1} of N, 5160 mg kg^{-1} of K, 1265 mg kg^{-1} of P, 1625 mg kg^{-1} of Ca, 1123 mg kg^{-1} of Mg, 90.6 mg kg^{-1} of Fe, and 23 mg kg^{-1} of Zn. The soil was inoculated with 100 spores of *C. claroideum* prior to sowing. After surface sterilization with 0.1% $HgC1_2$ (w/v) solution for 5 min, the maize seeds were sown in each pot at 8 cm depth. After 2 months of growth, when the AM fungus colonization level was 90%, shoots of maize were cut and the remaining root parts were uprooted. The roots along

with the soil in the pot, were taken as the AM inoculum and it contained spores, mycelium and root fragments.

Root colonization analysis

Root segments (1 cm length) of collected samples were first washed thoroughly in distilled water and then placed in 10% KOH and heated to 90 °C for 15–30 min. Root colonization was confirmed by the method of Phillips and Hayman (1970). Cleared roots were stained using 0.01% of trypan blue. A minimum of 100 root segments for each sample was examined for the assessment of percentage colonization of AM using the following formula,

$$\text{Root colonization}\,(\%) = \frac{\text{Number of segments colonized with AM}}{\text{Total number of segments analyzed}} \times 100$$

Fresh weight (FW) and dry weight (DW)

The fresh weight of the plants was directly measured using an electronic weighing balance. For dry weight measurements, the weighed samples were dried at 100 °C for 1 h and followed by 60 °C in a hot air oven until the weight attained a constant value.

Tissue moisture content %

The moisture content of leaves and roots was determined by measuring the fresh and dry weights of the leaves. Leaf moisture content percentage (MC%) was calculated using the following equation (Lokhande et al. 2011).

$$\text{Moisture content}\,\% = [(\text{FW} - \text{DW})/\text{FW}] \times 100$$

Estimation of primary metabolites

Five hundred milligrams of leaf and root tissue was homogenized in 5 mL of 0.1 M phosphate buffer (pH 7), and the soluble protein content of the plant material was estimated using Bradford reagent (Bradford 1976). The protein content in the shoot and root of *Z. mays* was expressed in mg g^{-1} FW.

Total soluble sugar content was extracted from leaf samples using 5 mL of 80% ethanol and estimated by Dubois et al. (1956). The standard curve was plotted with D-glucose as standard. The soluble sugar content in the shoot and root of *Z. mays* was expressed in mg g^{-1} FW.

Free amino acids were extracted from leaf samples using 80% ethanol, and estimation of the same was carried out following the method of Moore and Stein (1948) using ninhydrin reagent. Total free amino acids were calculated from a standard curve prepared with glycine. The free amino acids content in the shoot and root of *Z. mays* was expressed in mg g^{-1} FW.

Free proline content was extracted from shoot and root of maize plants using 3% sulphosalicylic acid and estimated following the method of Bates et al. (1973) using L-proline as standard. The free proline content in the shoot and root of *Z. mays* was expressed in μg g^{-1}FW.

Estimation of secondary metabolites

Total phenolics content was extracted using 80% ethanol and estimated according to the procedure described by Folin and Denis (1915) using Folin–Ciocalteau reagent (0.5 mL of 1 N). A standard curve was prepared using different concentrations of catechol. The phenolics content in the shoot and root of *Z. mays* was expressed in mg g^{-1} FW.

Two hundred milligrams of fresh leaf samples were homogenized in a clean mortar and pestle with 5 mL of the solvent containing acidified methanol (methanol: HCl: H_2O in 79:1:20). After 24 h, the estimation of flavonoids content was performed according to Mirecki and Teramura (1984). The flavonoids content in the shoot and root of *Z. mays* was expressed in mg g^{-1} FW.

Fresh leaf and root samples (0.2 g) were homogenized and extracted in 5 mL of acidified methanol (1:99, HCl: methanol, v/v). Further, the anthocyanin content was determined according to the method of Mancinelli et al. (1975). The anthocyanin content in the shoot and root of *Z. mays* was expressed in mg g^{-1} FW.

Three hundred milligrams tissue was homogenized with dimethyl sulphoxide (DMSO), and the alkaloid content was measured according to Talluri et al. (2018) using bromocresol green. The alkaloid content in the shoot and root of *Z. mays* was expressed in mg g^{-1} FW.

Five hundred milligrams of leaf tissue was homogenized with 5 mL of 5% (w/v) TCA and centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatant was collected, and the estimation of ascorbic acid (ASA) content was done according to the method of Chen and Wang (2006). The ascorbic acid content in the shoot and root of *Z. mays* was expressed in mg g^{-1} FW.

For the GC-MS analysis, the roots and leaves of mycorrhizal and non mycorrhizal maize plants subjected to Cd and Zn stressors were collected and washed with water and shade dried at room temperature. Extracts were prepared by the method of Grover and Patni (2013) using methanol as the solvent. The identification of metabolites in the samples was carried out using GC-MS (Shimadzu, QP2010, Kyoto, Japan). A Rxi-5Sil MS column (30 m length × 0.25 mm ID × 0.25 μm thickness) was used for gas chromatographic separation. Initially, the column temperature was 80 °C for 4 min, increased to 260 °C, and held for

6 min. The identity of the components in the extracts was assigned by comparing their retention indices and mass spectra fragmentation patterns with those stored in the computer library (NIST 11 and WILEY 8).

Fourier transform infrared (FT-IR) spectroscopic analysis of lignin

Isolation of lignin

Fourier Transform Infrared (FT-IR) spectroscopic analysis of lignin was performed according to the protocol of Domínguez-Robles et al. (2017). Two hundred milligrams of dried leaf and root samples were cut into 1 × 1 mm size and immersed in 7% NaOH, and heated at 100 °C for 150 min. After this pulping process, lignin isolation was performed by acid precipitating the dissolved lignin using a concentrated solution (95%) of sulfuric acid. After lowering the pH, solutions were kept for 24 h to allow the sedimentation of the precipitated lignin. To isolate the precipitated lignin, the samples were centrifuged at 8000 rpm for 20 min. Then the precipitates were washed with distilled water twice to discard possible impurities such as sugars or inorganic particles; finally, the samples were dried at 60 °C in an oven for 48 h.

Characterization of lignin

For the characterization of lignin, samples were mixed with potassium bromide (KBr) in a ratio of 1:150 mg (sample: KBr) with 10 tons of hydraulic pressure. FTIR analysis of the samples was carried out at midinfra-red region of 400–4000 cm^{-1} (Jasco 4100, Shanghai, China).

Estimation of bioaccumulated Zn and Cd

The leaves of maize plants were harvested and dried in an oven at 100 °C for 1 h and then at 60 °C until a constant weight was achieved. The same was used for Zn and Cd bioaccumulation studies according to the method of Allan (1969). From each sample, 1 g was digested by refluxing in 5:3 ratio of nitric:perchloric acid until the solution became colourless using Kjeldahl flask heated (60 °C) in a heating mantle. Subsequently, the digest was transferred to a standard flask, and the volume was made up to 100 mL. Atomic absorption spectrophotometer (AA-7000, Shimadzu, Kyoto, Japan) was used to estimate Zn and Cd present in the digested samples, and the concentration of metal in plants tissue was expressed in mg g^{-1} DW. The translocation factor (TF) of Cd and Zn was determined using the equation,

$$\text{Translocation factor (TF)} = \frac{\text{Concentration of metal in shoot}}{\text{Concentration metal in root}}$$

Statistical analysis

Statistical analysis of the results was carried out according to Duncan's multiple range tests at a 5% probability level. Data were subjected to one-way ANOVA using the SPSS software 16.0. Pearson's correlation analysis was performed to evaluate the relationships between the most important variables obtained in *Z. mays* under Cd and Zn toxicity. The data is an average from three independent experiments, each with three replicates. The data represent mean ± standard error. The biocomponents with a percentage higher than 1% of the total oil were subjected to a hierarchical cluster analysis (HCA) using SPSS v20.0 software. In the case of HCA, the dendrogram (tree) was produced using Ward's method of hierarchical clustering with squared Euclidean distance between the samples.

Results

AM root colonization

The percentage of AM root colonization was increased to 90–100% in the roots of maize plants on 45 d of growth (Fig. 1) which was confirmed by the presence of different structures like arbuscules, vesicles along with the extensive ramification of fungal hyphae. At the same time, non AM plants did not show any mycorrhizal structures in the root.

Selection of stress imparting concentrations of Cd and Zn

Zea mays was exposed to different concentrations of $CdCl_2$ (0, 0.225, 0.45, 0.675 g Cd Kg^{-1} soil) and $ZnSO_4$ (0, 0.65, 1.30, 1.95, 2.62 g Zn Kg^{-1} soil). Of these, 0.45 g Cd Kg^{-1}soil (as $CdCl_2$) and 1.95 g Zn Kg^{-1} soil (as $ZnSO_4$) were selected as stress imparting concentrations as these concentrations caused ~50% growth reduction in plants on exposure to Cd and Zn stresses on 8 d. The 50% growth reduction was determined based on the enhancement in the MDA content and reduction in the total chlorophyll and tissue moisture contents (Supplementary Fig. 1). Treating *Z. mays* with lower concentrations of Zn (0.65, 1.30 g Kg^{-1} soil) and Cd (0.225 g Kg^{-1} soil) does not impose a stress condition when compared to the control plants on 8 d of heavy metal exposure. Conversely, higher concentrations of Zn (2.62 g Kg^{-1} soil) and Cd (0.675 g Kg^{-1} soil) were found to be detrimental to the plants as they developed symptoms of severe wilting upon the increase in the treatment period (12 d). Hence for the evaluation of mycorrhizal mediated heavy metal stress tolerance

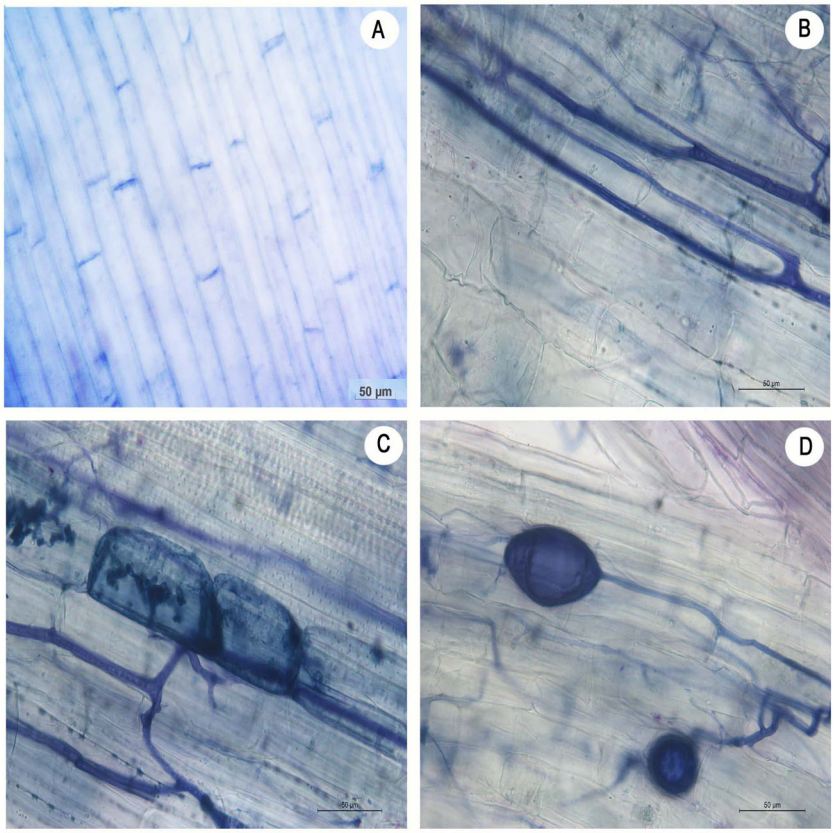

Fig. 1 Micrographs of maize, non AM plants (**A**) and AM plants (**B**, **C**, **D**)

potential in *Z. mays*, the soils were supplied with 0.45 g Cd Kg^{-1} and 1.95 g Zn Kg^{-1} soil.

Fresh weight and dry weight

Mycorrhizae associated *Z. mays* showed a significant increase in the fresh weight of both shoots and roots as compared to non AM plants in optimal conditions, but on exposure to heavy metal stress, the fresh weight was reduced. On exposure to Cd stress, non AM and AM plants of *Z. mays* showed 73 and 40% reduction in fresh weight as compared to the control plants. Zn caused a 56% reduction in the fresh weight of non AM plants, but Zn did not significantly reduce the fresh weight of AM plants (Table 1). In the case of *Z. mays*, root fresh weight was increased, and the increase was insignificant in non AM plants, but in AM plants, the increase under Cd and Zn toxicity was 211–212% (Table 1).

Mycorrhizal association increased the dry weight of *Z. mays* in optimal condition, but the exposure to heavy metal stress decreased the dry weight. In the shoot of non AM plants of *Z. mays*, Cd and Zn induced reduction in the dry weight, which was insignificant on 12 d of treatment. However, the dry weight of the mycorrhizal plants increased to the extent of 86–145% as compared to the control, even under metal stress. In the root, the dry weight was reduced to 49–50% in non AM plants exposed to Cd and Zn stress. But the root dry weight showed an 8–9 fold increase in the mycorrhizal roots exposed to Cd and Zn stressors compared to the control (Table 1).

Tissue moisture content percentage

Heavy metal treatment significantly reduced the moisture content of the shoot system. The shoot tissue moisture content of *Z. mays* showed 32–37% reduction in non AM plants under Cd and Zn stresses, but the reduction was less in AM plants. On exposure of plants to Cd and Zn, the root moisture content was slightly increased in non AM plants, but it was decreased in AM plants (Table 1).

Primary metabolites

Primary metabolites such as soluble sugar, proteins, amino acids, and proline content were significantly altered in the shoots and roots of non AM as well as AM plants on exposure to Cd and Zn stresses. Both Cd and Zn stressors augmented the soluble sugar content in the leaves of non AM and AM plants (Fig. 2A) on initial days of exposure. The increase was 132–167% in Cd and Zn treated non AM plants and AM plants on the 8 d of exposure. Further, the

Table 1 Heavy metal-induced changes in the fresh weight, dry weight and moisture content of non AM and AM plants of *Z. mays*

Days	Treatments	Shoot			Root		
		Fresh weight (g)	Dry weight (g)	Moisture content %	Fresh weight (g)	Dry weight (g)	Moisture content %
0 d	Non AM	4.433 ± 0.575^{d}	0.373 ± 0.065^{e}	91.301 ± 1.719^{a}	1.287 ± 0.338^{c}	0.058 ± 0.012^{b}	94.818 ± 0.999^{a}
	AM	6.053 ± 0.66^{b}	0.843 ± 0.024^{c}	85.778 ± 1.422^{c}	5.413 ± 0.122^{a}	0.627 ± 0.026^{a}	88.397 ± 0.708^{b}
	Non AM + $CdCl_2$	4.673 ± 0.08^{d}	0.473 ± 0.018^{d}	95.273 ± 0.528^{a}	1.287 ± 0.327^{c}	0.063 ± 0.012^{b}	94.121 ± 1.971^{a}
	AM + $CdCl_2$	7.82 ± 0.587^{a}	0.927 ± 0.076^{b}	87.99 ± 1.392^{b}	5.08 ± 0.327^{a}	0.617 ± 0.026^{a}	87.61 ± 0.559^{b}
	Non AM + $ZnSO_4$	5.3 ± 0.587^{c}	0.43 ± 0.191^{d}	88.034 ± 3.526^{b}	1.62 ± 0.666^{c}	0.063 ± 0.012^{b}	94.357 ± 2.207^{a}
	AM + $ZnSO_4$	7.72 ± 0.212^{a}	0.97 ± 0.078^{a}	87.393 ± 1.214^{b}	4.747 ± 0.433^{b}	0.627 ± 0.026^{a}	86.578 ± 1.412^{b}
4 d	Non AM	4.767 ± 0.332^{b}	0.383 ± 0.061^{d}	92.043 ± 0.734^{b}	1.287 ± 0.338^{d}	0.063 ± 0.012^{d}	94.818 ± 0.999^{a}
	AM	6.387 ± 0.354^{a}	0.67 ± 0.078^{a}	84.752 ± 1.336^{c}	5.413 ± 0.122^{a}	0.66 ± 0.044^{a}	87.8 ± 0.823^{b}
	Non AM + $CdCl_2$	3.153 ± 1.115^{c}	0.39 ± 0.016^{cd}	81.821 ± 8.833^{c}	0.917 ± 0.105^{e}	0.0727 ± 0.015^{c}	92.466 ± 4.488^{a}
	AM + $CdCl_2$	5.923 ± 0.471^{a}	0.417 ± 0.217^{c}	92.662 ± 3.679^{b}	4.677 ± 0.444^{b}	0.447 ± 0.028^{b}	$90.304 \pm 0.959a$
	Non AM + $ZnSO_4$	3.317 ± 0.508^{c}	0.347 ± 0.032^{e}	95.359 ± 1.159^{a}	0.873 ± 0.092^{e}	0.093 ± 0.012^{c}	96.862 ± 2.274^{a}
	AM + $ZnSO_4$	6.037 ± 0.136^{a}	0.567 ± 0.266^{b}	90.565 ± 4.377^{c}	4.547 ± 0.325^{b}	0.47 ± 0.071^{b}	91.542 ± 2.333^{a}
8 d	Non AM	4.4333 ± 0.575^{c}	0.323 ± 0.015^{c}	92.502 ± 0.84^{a}	1.287 ± 0.338^{d}	0.063 ± 0.012^{c}	94.818 ± 0.999^{a}
	AM	6.0533 ± 0.66^{a}	0.627 ± 0.024^{b}	82.644 ± 1.939^{b}	5.413 ± 0.122^{a}	0.627 ± 0.026^{a}	88.397 ± 0.708^{b}
	Non AM + $CdCl_2$	1.1533 ± 0.123^{e}	0.309 ± 0.016^{d}	65.649 ± 2.657^{d}	1.517 ± 0.176^{c}	0.043 ± 0.107^{d}	97.043 ± 1.688^{a}
	AM + $CdCl_2$	2.59 ± 0.227^{d}	0.617 ± 0.065^{b}	75.436 ± 4.552^{c}	4.343 ± 0.187^{b}	0.42 ± 0.006^{b}	90.303 ± 0.33^{b}
	Non AM + $ZnSO_4$	1.983 ± 0.402^{e}	0.263 ± 0.006^{e}	64.718 ± 9.015^{d}	1.454 ± 0.097^{c}	$0.035 \pm 0.009d$	97.834 ± 0.756^{a}
	AM + $ZnSO_4$	4.947 ± 0.12^{b}	0.69 ± 0.121^{a}	81.889 ± 2.092^{b}	4.347 ± 0.268^{b}	0.437 ± 0.065^{b}	89.866 ± 1.718^{b}
12 d	Non AM	3.767 ± 0.332^{c}	0.313 ± 0.015^{d}	91.351 ± 0.422^{a}	1.287 ± 0.338^{d}	0.063 ± 0.012^{d}	94.818 ± 0.999^{a}
	AM	5.387 ± 0.478^{a}	0.887 ± 0.05^{a}	81.45 ± 1.66^{b}	5.413 ± 0.122^{a}	0.627 ± 0.026^{a}	88.397 ± 0.708^{b}
	Non AM + $CdCl_2$	0.987 ± 0.207^{e}	0.283 ± 0.064^{de}	61.625 ± 13.919^{d}	1.297 ± 0.2^{d}	0.031 ± 0.06^{e}	97.603 ± 10.851^{a}
	AM + $CdCl_2$	2.257 ± 0.532^{c}	0.583 ± 0.095^{c}	67.892 ± 13.731^{c}	4.01 ± 0.268^{b}	0.537 ± 0.124^{c}	86.841 ± 2.204^{b}
	Non AM + $ZnSO_4$	1.65 ± 0.437^{d}	0.263 ± 0.006^{e}	57.062 ± 9.285^{d}	1.347 ± 0.088^{c}	0.032 ± 0.215^{e}	97.6218 ± 19.83^{a}
	AM + $ZnSO_4$	3.613 ± 0.436^{b}	0.767 ± 0.098^{b}	83.226 ± 2.012^{b}	4.013 ± 0.159^{b}	0.603 ± 0.105^{b}	89.785 ± 3.114^{b}

Values are expressed as mean ± SE of three independent experiments. Different letters superscript to the values indicate a significant difference between treatments (Duncan's test, $p \leq 0.05$). Where BDL indicates below detectable level

sugar content was reduced (28–34%) in non AM plants as compared to the control, whereas the AM plants maintained the sugar content in the leaves even on the 12 d of exposure to Cd and Zn stressors. The sugar content was decreased in roots of non AM and AM plants on the 12 d of exposure to Cd and Zn stressors (Fig. 2B). The reduction was to the extent of 92–86% in non AM plants but was only 35–44% in AM plants under metal stress.

Both Cd and Zn stressors induced an increase in the protein content in the leaves of non AM and AM plants (Fig. 2C). Non AM plants had recorded 72 and 48% enhancement in protein content under Cd and Zn stressors respectively as compared to the control, whereas the increase was 39 and 73% in AM plants under Cd and Zn stressors on 8 d. Contrary to that of leaves, the protein content was reduced in roots of non-AM and AM plants of *Z. mays* subjected to both Cd and Zn stresses (Fig. 2D). On 12 d of Cd stress, the protein content was insignificantly reduced in non-AM plants; at the same time, there was an insignificant increase in AM plants. But the reduction was 53 and 11% in the roots of non-AM and AM plants on 12 d exposure of Zn stress.

Cd and Zn stressors elicited amino acids accumulation in the leaves of non AM and AM plants (Fig. 2E). The increase was 92 and 206% in Cd and Zn treated non AM plants, but it was only 34 and 40% in AM plants on 4 d of exposure. Furthermore, on 12 d of stress, the amino acids content was decreased to the extent of 58% in Cd treated non AM plants, but not in AM plants. In roots, the amino acids content was decreased in non-AM plants of *Z. mays* on exposure to Cd and Zn stresses, and it was to the extent of 64–65%, but the reduction was only 13 and 26% in Cd and Zn treated AM plants (Fig. 2F).

Both Cd and Zn stressors induced an increase in the proline content in the leaves of non AM and AM plants (Fig. 2G). The enhancement in proline content was up to 4 and 21 fold in non-AM plants under Cd and Zn stresses respectively, as compared to the control. But, in the leaves of AM plants, only 44–64% increment in proline

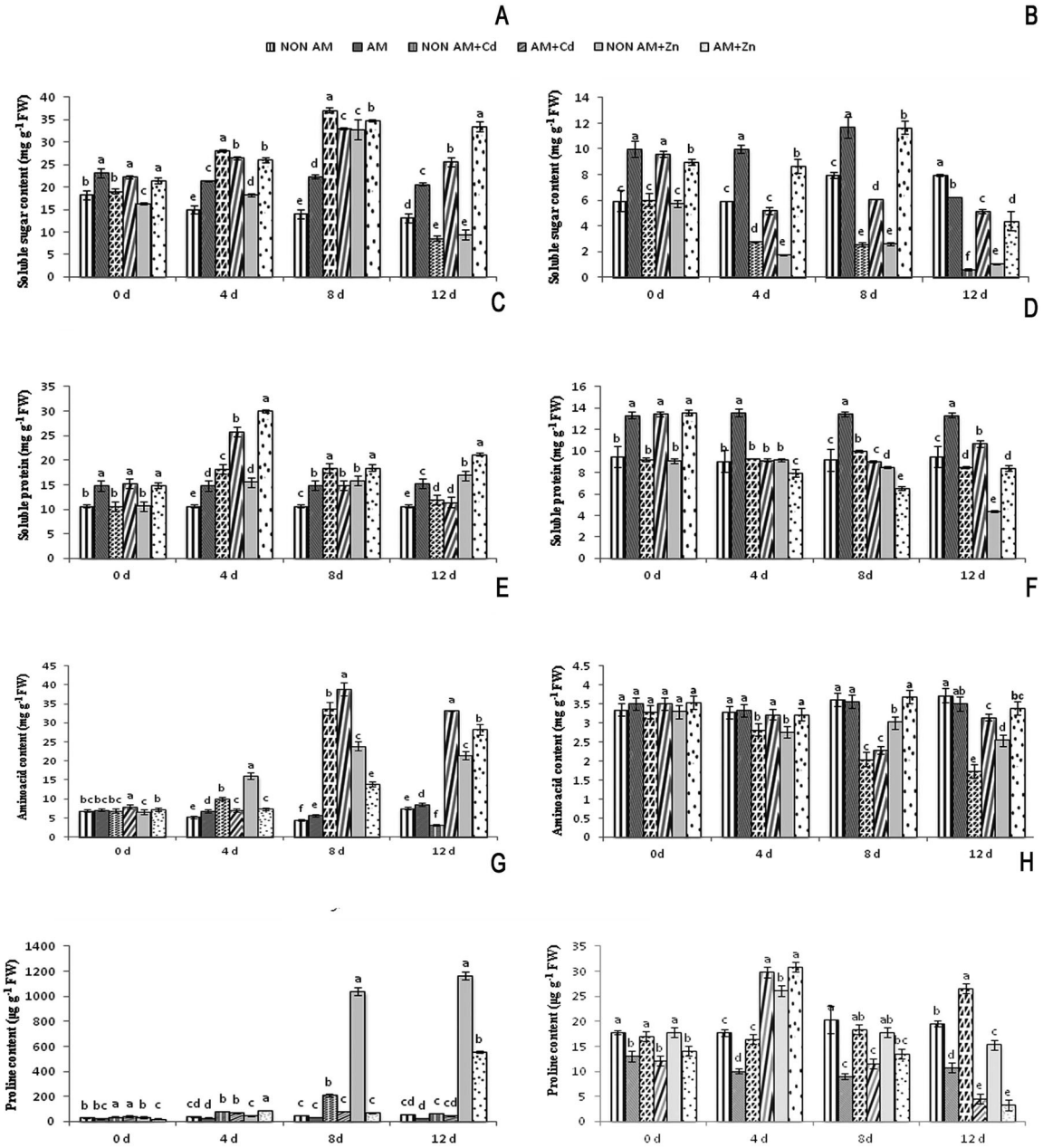

Fig. 2 Alterations observed in primary metabolite accumulation of maize plants associated with mycorrhiza exposed to $ZnSO_4$ and $CdCl_2$; total soluble sugar content in the leaves (**A**) and roots (**B**), total soluble protein content in the leaves (**C**) and roots (**D**), total free amino acid content in the leaves (**E**) and roots (**F**), and total proline content in the leaves (**G**) and roots (**H**) of HM treated and control plants

content occurred on 8 d of Cd and Zn stresses. Contrary to that of shoots, proline content was reduced in roots of *Z. mays*, the reduction in proline content was 33–43% in Cd and Zn treated non-AM plants, whereas the reduction was only 9–12% in AM associated roots (Fig. 2H). Total proline content showed a strong significant positive correlation with sugar content in the leaves of AM plants ($r = 0.932$, $p \leq 0.05$), but it was negative and insignificant in non AM ($r = -0.310$, $p \geq 0.05$) plants exposed to Cd and Zn toxicity (Supplementary Table 1).

Secondary metabolites

Phenolics content was increased to the extent of 59 and 84% in the leaves of non AM plants on exposure to Cd and Zn respectively. The increase was only 44% in Cd treated, and it was insignificant in Zn treated AM plants on 4 d of treatment (Fig. 3A). However, this trend of phenolics accumulation changed with the extension of days of exposure, and AM plants accumulated more phenolics content on 8 d of exposure to Cd and Zn

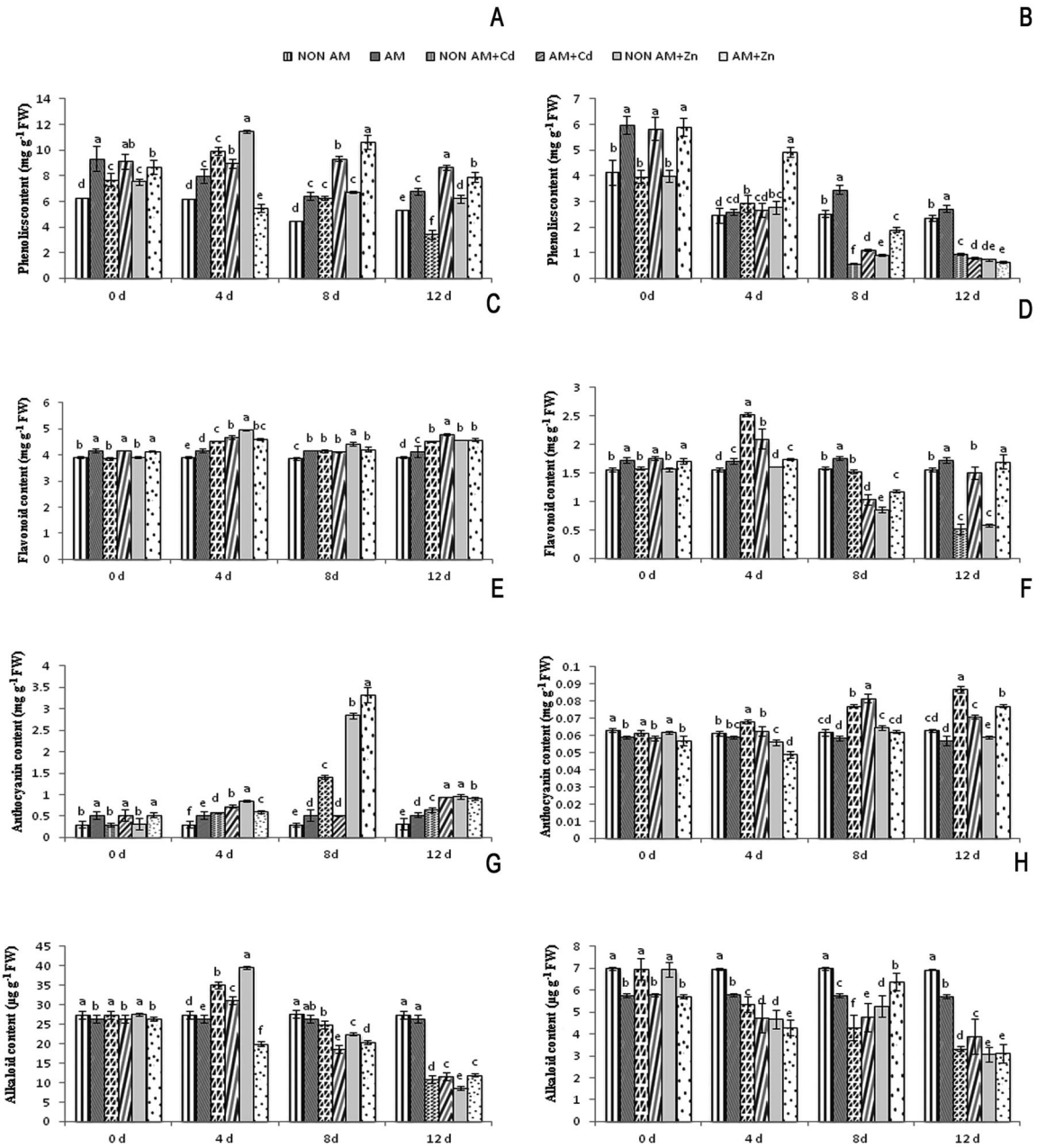

Fig. 3 Alterations observed in secondary metabolite accumulation of maize plants associated with mycorrhiza exposed to $ZnSO_4$ and $CdCl_2$; total phenolics content in the leaves (**A**) and roots (**B**), total flavonoid content in the leaves (**C**) and roots (**D**), anthocyanin content in the leaves (**E**) and roots (**F**), and alkaloid content in the leaves (**G**) and roots (**H**) of HM treated and control plants

stressors. Phenolics content was decreased in roots of non AM and AM plants on exposure to Cd and Zn (Fig. 3B). The reduction was 77 and 64% in Cd and Zn treated non AM plants, and the reduction was only 56 and 24% in AM plants as compared to the control on 8 d of exposure. Total phenolics content had a strong significant positive correlation with amino acids content in the leaves of non AM plants ($r = 0.870$, $p \leq 0.05$) and in AM ($r = 0.947$, $p \leq 0.05$) plants exposed to Cd and Zn toxicity.

Both Cd and Zn stressors induced an increase in the flavonoids content in the leaves of non AM and AM plants (Fig. 3C). Non AM plants showed 15–26% enhancement in flavonoids content under Cd and Zn stress as compared to the control. But, in the leaves of AM plants, only 17–19% increment in flavonoids content occurred on 4 d of Cd and Zn treatment. Contrary to that of shoots, both Cd and Zn stressors reduced the flavonoids content in roots of non AM and AM plants (Fig. 3D). The reduction in flavonoids content was 62–67% in roots of Cd and Zn treated non AM

Table 2 The bioactive compounds detected in leaves of *Z. mays* plants associated with mycorrhiza, exposed to $ZnSO_4$ and $CdCl_2$ stress

Bioactive compounds	Non AM	AM	Non AM + Cd	AM + Cd	NonAM + Zn	AM + Zn
1,2-benzenedicarboxylic acid, diisooctyl ester	–	1.87	–	–	–	–
3β-acetoxystigmasta-4,6,22-triene	–	–	3.35	–	–	–
B-linalool	–	–	–	2.47	–	–
Cholesta-4,6-dien-3-ol, benzoate, (3β)-	–	2.34	5.48	2.18	3.31	3.86
γ-sitosterol	–	–	7.1	10.72	–	–
Hahnfett	6.96	3.09	6.76	9.49	2.47	1.4
Hexadecadienoic acid, methyl ester	13.05	2.58	–	–	–	7.83
8-hydroxylinalool	–	–	3.75	–	–	–
Methyl linolenate	2.52	2.52	–	2.56	2.81	4.42
Methyl palmitate	2.41	7.49	5.04	6.23	3.6	4.1
Methyl stearate	–	1.49	1.51	2.59	1.04	1.04
Neophytadiene	24.21	25.16	12.01	14.1	21.71	20.43
Octadecedien-1-ol	–	–	–	–	2.37	4.53
Octadecadienoic acid, methyl ester	–	3.62	1.18	–	–	1.05
1-Oxacyclopentadecan-2-one, 15-ethenyl-15-methyl	–	–	1.73	–	–	–
Palmitate	–	2.58	–	–	15.68	–
2-Pentadecanone, 6,10,14-trimethyl-	2.97	2.21	5.8	4.46	4.81	7.2
(E)-phytol	11.81	9.12	4.91	7.68	16.49	17.15
Phytol	26.96	17.06	23.41	31.11	13.8	13.72
Squalene	1.25	0.86	1.56	1.86	1.01	–
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	7.86	15.53	8.03	4.56	9.22	9.36

plants, whereas the reduction was insignificant in AM associated roots on 12 d of metal treatment.

Cd and Zn stressors elicited anthocyanin accumulation in the leaves of non AM and AM plants (Fig. 3E). The increase was 4 and 9 fold in Cd and Zn treated non AM plants, and it was 1 and 10 fold in AM plants on 8 d of exposure. Anthocyanin content was increased in the roots of non AM to the extent of 25% in Cd-treated plants, and the increase was only 32% in AM plants exposed to Cd (Fig. 3F). Zn toxicity did not induce any significant change in the anthocyanin content of roots on 8 d of exposure. Total anthocyanin content showed a strong significant positive correlation with proline content in the leaves of non AM plants ($r = 0.855$, $p \le 0.05$), but it was insignificant in AM ($r = 0.480$, $p \ge 0.05$) plants exposed to Cd and Zn toxicity. Similarly, total anthocyanin content had a significant positive correlation with total amino acid content in the leaves of non AM ($r = 0.717$, $p \le 0.05$) and AM plants ($r = 0.992$, $p \le 0.05$) exposed to Cd and Zn toxicity. But the total anthocyanin content of non AM plants and AM plants were differentially correlated with the sugar content, and it had a significant negative correlation in non AM plants ($r = -0.731$, $p \le 0.05$) and a significant positive correlation in AM plants ($r = 0.702$, $p \le 0.05$)

Cd and Zn toxicity increased the alkaloid content in the leaves of non AM plants to the extent of 27 and 42%. In AM plants, the enhancement in alkaloid content under Cd stress was only 13%, and it decreased (27%) under Zn treatment on 4 d of exposure (Fig. 3G). Both Cd and Zn stressors reduced the alkaloid content in roots of non AM and AM plants (Fig. 3H). The reduction in alkaloid content was 24–38% in Cd and Zn treated non AM plants, whereas the reduction was only 8–31% in AM associated roots.

Bioactive compounds

Cadmium and zinc-induced modulation in the phytochemical composition in leaves of non AM and AM plants is compiled in Table 2. Neophytadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, (E)-phytol, methyl palmitate, 3,6-octadecadienoic acid, squalene, hahnfett, linolenic acid methyl ester, and phytol were the different bioactive compounds detected generally in the leaves of non AM and AM plants. Whereas 1,2-benzenedicarboxylic acid and cholesta-4,6-dien-3-ol, benzoate, (3β)- were specific to the leaves of mycorrhizal plants. Cd-induced the accumulation of γ-sitosterol, 3β-acetoxystigmasta-4,6,22-triene and cholesta-4,6-dien-3-ol, benzoate, (3β) in non-mycorrhizal plants. At

Table 3 The difference in the phytol composition in leaves of maize plants associated with mycorrhiza, exposed to $ZnSO_4$ and $CdCl_2$ stress

Treatments	Phytol (%)	(E)-phytol (%)	Phytol/(E)-phytol
NON AM	26.96	11.81	2.28
AM	17.06	9.12	1.87
NON AM + $CdCl_2$	23.41	4.91	4.77
AM + $CdCl_2$	31.11	7.68	4.05
NON AM + $ZnSO_4$	13.8	16.49	0.84
AM + $ZnSO_4$	13.72	17.15	0.80

the same time, Cd elicited the production of β-linalool and γ-sitosterol in mycorrhizal plants. Exposure to $CdCl_2$ also reduced the area percentage of neophytadiene in mycorrhizal and non-mycorrhizal plants.

As compared to the control plants, Zn induced the accumulation of octadecadien-1-ol and cholesta-4,6-dien-3-ol, benzoate in AM and non AM plants. Exposure to $ZnSO_4$ also caused a modification in the ratio between phytol and (E)-phytol. Two forms of phytol with two different retention times (Rt) were detected in the methanolic extract of maize leaves, (E)-Phytol (Rt-27.387) and phytol (Rt-31.849). In the present study, $CdCl_2$ altered the ratio between phytol and (E)-phytol in both mycorrhizal and non-mycorrhizal plants (Table 3). The alteration in the ratio between phytol and (E)-Phytol was significant, and it was metal-dependent. In non AM and AM plants, the ratio was 1.8–2.2, but under Cd toxicity, it was increased to 4 in both non AM and AM plants. When the non AM and AM plants were exposed to Zn toxicity, the ratio between phytol and (E)-Phytol was reduced to 0.8.

Mycorrhizae and heavy metal-induced alterations in the chemical constituents were clearly observed in the root (Table 4). 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, 1,2-benzenedicarboxylic acid, γ-sitosterol, eicosane, tetracosanoic acid, squalene, nonadecane, n-nonadecanol-1, methyl stearate, methyl palmitate, methyl isopimarate, methyl 9,12-octadecadienoate, glycerol β-palmitate, longifolenbromid-I, diethylene glycol dibenzoate, cholesta-4,6-dien-3-ol, (3β.)-, 3β-acetoxystigmasta-4,6,22-triene, 10-octadecenoic acid, methyl ester are the compounds detected in the roots of non AM plants. Different from this, 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, 10-octadecenoic acid, 4,22-stigmastadiene-3-one, 9,19-cyclolanost-24-en-3-ol, (3β)-, 9,19-cyclolanost-24-en-3-ol, (3β)-, cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate, stigmasta-5,22-dien-3-ol, (3β,22e)-, oleic acid, propyl ester, octadecane, methyl palmitate, methyl 9,12-octadecadienoate, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, heptadecane were the compounds observed in mycorrhizal roots.

Cd and Zn also induced significant alterations in the phytochemistry of root, 16-β-hydroxydigitoxigenin, elixene, linoleic acid, methyl ester, hexahydrofarnesyl acetone, methyl melissate are the Cd-induced compounds detected in the maize roots. Furthermore, mycorrhization induced the production of retinol, acetate, pentacosane, oxalic acid, heptadecyl hexyl ester, oleic acid, methyl ester, l-norvaline, n-(2-methoxyethoxycarbonyl)-, hexadecyl ester in maize roots on exposure to Cd. The metal-dependent phytochemical modification was observed in the root, which was evidenced by the detection of new biomolecules in the roots as a result of Zn toxicity. 2-pentadecanone, 6,10,14-trimethyl-, cycloartenol, eicosanoic acid, methyl ester, longifolenbromid-I, solanesol are the Zn induced compounds observed in non mycorrhizal plants. Different from the non AM plants, mycorrhizal association induced the production of 4,22-Stigmastadiene-3-one, eicosane, 9,19-cyclolanost-24-en-3-ol, (3β)-heptadecane, hexahydrofarnesyl acetone in the maize roots exposed to Zn toxicity.

From the dendrogram produced by HCA, the shoot system of heavy metal-treated maize can be classified into two main clusters at a distance of 25 units (Fig. 4A). The essential oil profile of samples connected by a shorter distance is more similar than those connected by a longer distance. The first cluster (cluster 1) is represented by Cd treated non AM and AM plants, and it indicates metal-specific modifications in the essential oil composition of the shoot system. The second cluster (cluster 2) represents four groups, including non AM, AM, Zn treated non AM and AM plants. It indicates the Zn treated samples showed less deviation from the essential oil composition of non AM and AM plants.

But in the case of root, where mycorrhization can directly influence the chemical composition, the grouping was entirely different (Fig. 4B). The dendrogram exhibited two main clusters in a distance of 25 units, and each cluster contained three samples. The first cluster (cluster 1) represented by non AM plants includes control, Cd treated and Zn treated plants, whereas Cd treatment does not induce a significant modification in the essential oil composition of the root as compared to the Zn treated roots. In the second cluster (cluster 2), AM plants including Cd and Zn treated plants were aligned together, and it points to the strong modifications in the essential oil composition elicited by mycorrhization. In mycorrhizal plants, Cd-induced significant modifications as compared to Zn and thus Zn treated AM plants were connected to AM plants by a shorter distance.

Characterization of lignin

The FT-IR spectra of the lignin samples from the shoots and roots of maize plants were recorded in the range of 4000–400 cm^{-1} (Figs. 5, 6).

Table 4 The bioactive compounds detected in the root of maize plants associated with mycorrhiza, exposed to $ZnSO_4$ and $CdCl_2$ stress

Bioactive compounds	Non AM	AM	Non AM + Cd	AM + Cd	NonAM +Zn	AM + Zn
Eicosane	2.53	–	–	12.07	–	1.97
γ-sitosterol	12.62	3.51	9.91	0.00	5.84	0.00
1,2-benzenedicarboxylic acid	12.41	10.31	10.61	11.48	10.82	5.13
1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	7.06	4.32	6.34	–	–	–
10-Methyl-10-nonadecanol	–	–	–	3.80	–	–
10-Octadecenoic acid, methyl ester	3.76	12.78	7.77	4.71	8.92	4.53
16-β-hydroxydigitoxigenin	–	–	4.56	0	0	0
1-heneicosanol	3.25	–	–	1.73	2.65	6.27
1-hexadecanol	–	–	–	–	0	2.75
2-pentadecanone, 6,10,14-trimethyl-	–	–	–	–	1.37	–
3β-acetoxystigmasta-4,6,22-triene	4.37	–	1.79	2.097	1.16	–
4,22-Stigmastadiene-3-one	–	7.04	3.73	–	–	12.41
9,19-Cyclolanost-24-en-3-ol, (3β)-	–	1.31	–	–	–	2.36
9-octadecenamide	–	–	–	4.9	3.64	2.22
Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate	–	2.65	–	–	–	–
Cholesta-4,6-dien-3-ol, (3.β.)-	2.64	–	1.68	–	–	–
Cycloartenol	0	–	–	–	1.59	–
Diethylene glycol dibenzoate	2.54	–	–	–	2.12	–
Eicosanoic acid, methyl ester	–	–	–	–	6.27	–
Elixene	–	–	2.22	-	0	–
Glycerol β-palmitate	9.21	–	8.94	1.98	11.71	–
Heptadecane	–	–	–	–	–	1.93
Hexadecane	–	1.21	–	–	1.52	–
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	–	16.22	–	–	–	–
Hexahydrofarnesyl acetone	–	–	2.77	–	–	1.66
Linoleic acid, methyl ester	–	–	5.77	–	–	–
L-Norvaline, N-(2-methoxyethoxycarbonyl)-, hexadecyl ester	–	–	–	2.71	–	–
Longifolenbromid-i	–	–	–	–	8.27	–
Methyl 9,12-octadecadienoate	5.42	6.01	–	–	–	2.4
Methyl isodextropimarate	–	–	–	–	–	8
Methyl isopimarate	1.38	–	–	1.82	–	–
Methyl lignocerate	–	–	–	3.98	–	3.85
Methyl melissate	–	–	1.77	–	–	–
Methyl palmitate	15.39	20.54	23.93	17.44	12.53	15.02
Methyl stearate	5.32	–	6.00	11.43	11.43	6.99
N-Nonadecanol-1	2.13	–	–	–	0	–
Nonadecane	–	–	–	–	1.81	2.13
Octadecane	–	1.42	–	3.61	–	–
Oleic acid, methyl ester	–	0	–	4.34	–	–
Oleic acid, propyl ester	–	6.33	–	–	–	–
Oxalic acid, heptadecyl hexyl ester	–	–	–	1.71	–	–
Pentacosane	–	–	–	3.86	–	–

Table 4 (continued)

Bioactive compounds	Non AM	AM	Non AM + Cd	AM + Cd	NonAM +Zn	AM + Zn
Phytol	–	–	2.2	–	–	3.44
Retinol, acetate	–	–	–	1.69	–	–
Solanesol	–	–	–	–	12.78	–
Squalene	3.74	–	–	4.65	–	–
Stigmasta-5,22-dien-3-ol, (3.β.,22e)-	–	7.04	3.73	–	–	12.41
Tetracosanoic acid, methyl ester	3.36	4.79	–	–	–	–
Trans-13-Octadecenoic acid, methyl ester	–	–	–	–	2.54	1.61

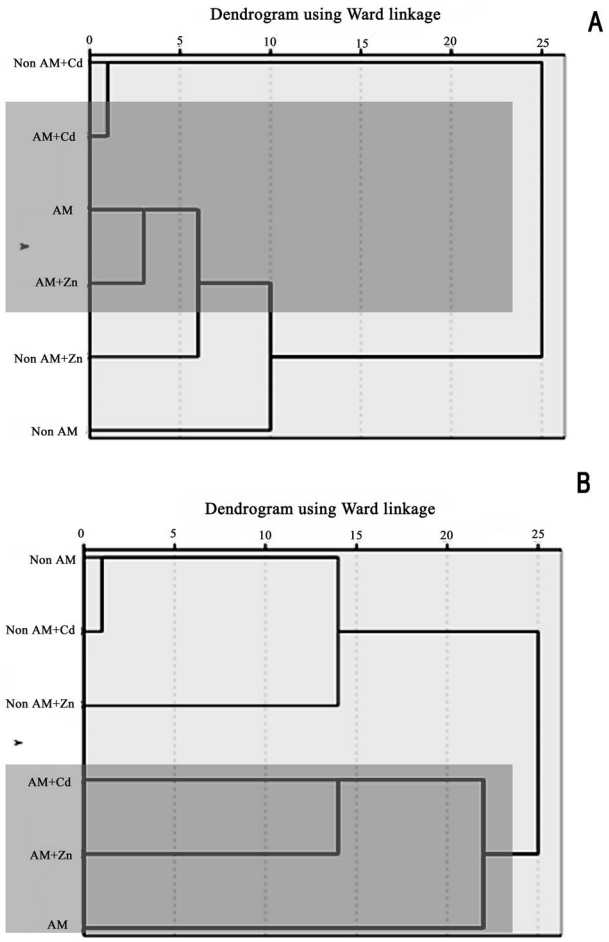

Fig. 4 Hierarchical cluster analysis of the biocompounds observed in maize plants associated with mycorrhiza exposed to $ZnSO_4$ and $CdCl_2$; dendrogram obtained in shoot (**A**) and root (**B**)

The shoot lignin samples showed almost similar spectroscopic patterns in control and plants under Cd and Zn stress. The peaks observed at 3403, 2911, 2846, 1716, 1624, 1466, 1395, 1322, 1285, 1233, 1178, 1067, 887, 849, 616, 575 and 458 cm^{-1} are the common peaks to the lignin isolated from the leaves of non mycorrhizal and mycorrhizal plants exposed to Cd stress (Fig. 5). However, compared to all other samples in Zn treated non AM and AM plants, the peaks at 2911 and 2846 were absent.

In roots, the basic spectroscopic patterns of lignin were almost the same in all six samples, but metal-treated plants showed some differences in the pattern (Fig. 6). 3403, 2911, 2846, 1716, 1624, 1466, 1395, 1322, 1285, 1233, 1178, 1067, 887, 849, 616, 575 and 458 cm^{-1} were observed in root lignin composition also. Three peaks at 1285, 1233, 1178 cm^{-1} were prominent in plants subjected to Cd and Zn stressors but were not shown in the non-treated non AM and AM plants.

Bioaccumulation of Cd and Zn

The pattern of Cd and Zn accumulation in maize roots and leaves was different, and the roots showed an increase in the metal accumulation as compared to the leaves of non AM and AM plants subjected to Cd and Zn treatments (Table 5). In the plants exposed to Cd stressor, maximum Cd ion accumulation was recorded in the roots of non AM plants (5.0488 ± 0.2524 mg g^{-1} DW) followed by AM plants (3.7446 ± 0.1872 mg g^{-1} DW) on 8 d of exposure. Leaves accumulate low concentrations of Cd as compared to roots; it was 0.6094 ± 0.0305 mg g^{-1} DW and 0.6525 ± 0.0326 mg g^{-1} DW in non AM and AM plants respectively on 8 d of exposure (Table 2). Under Zn stress also, maximum Zn accumulation was observed in the roots of non AM plants (0.7409 ± 0.037 mg g^{-1} DW) as compared to AM plants (0.251 ± 0.0013 mg g^{-1} DW). The Zn accumulation by the leaves was very low; it was 0.1222 ± 0.0061 mg g^{-1} DW in non AM plants and 0.0888 ± 0.0044 mg g^{-1} DW in AM plants. At optimal conditions, translocation factor (TF) of Zn was observed as 0.35 and 0.45 in non AM and AM plants respectively, but under Zn toxicity, it was reduced to 0.16 in non AM plants, and it was maintained as 0.35 in AM plants. Whereas the TF of Cd was 0.12 and 0.17 in non AM and AM plants respectively on 8 d of exposure to Cd stressor.

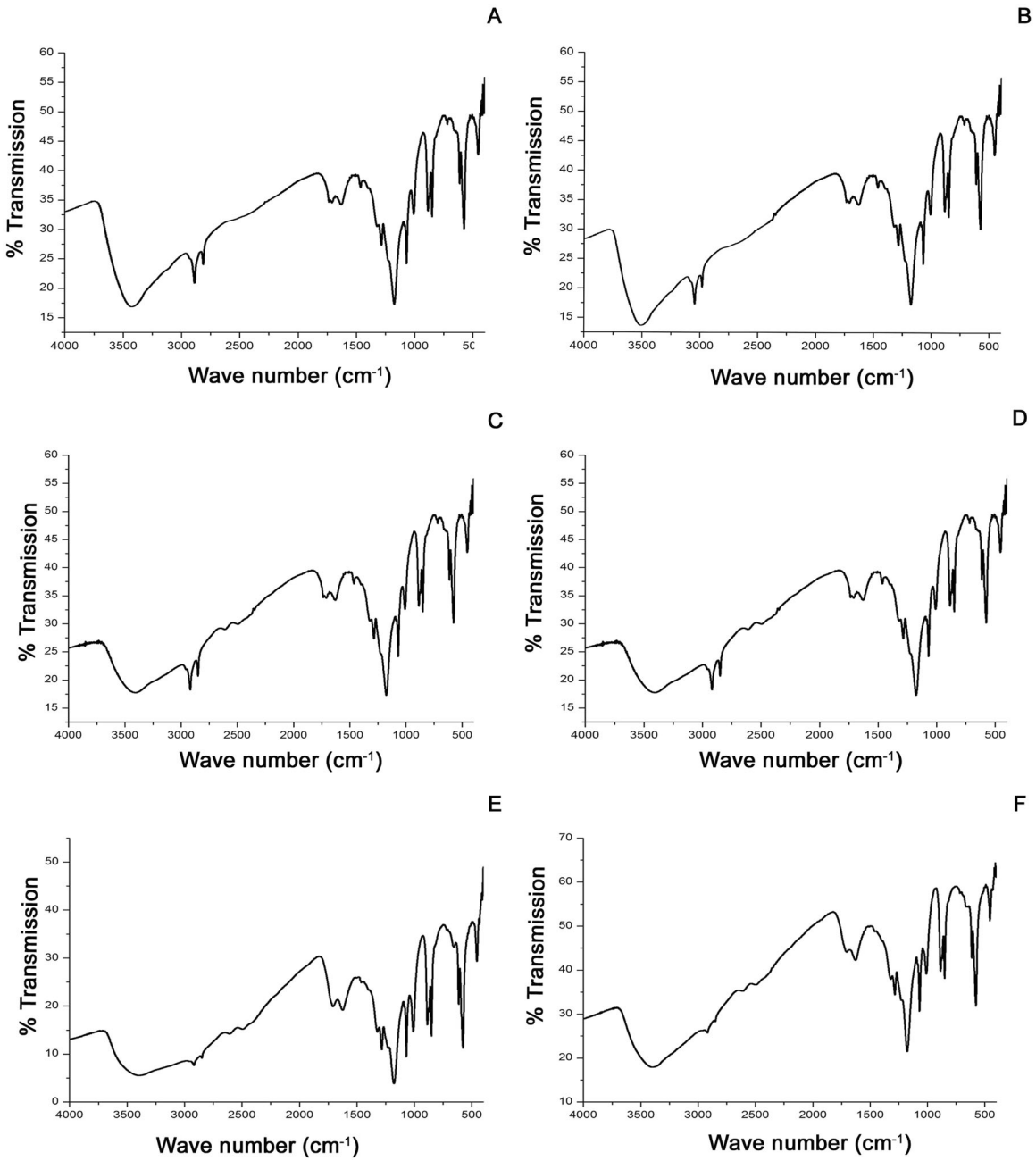

Fig. 5 Characterization of lignin isolated from the maize leaves associated with mycorrhiza exposed to $ZnSO_4$ and $CdCl_2$; FTIR spectrum of Non AM (**A**), AM (**B**), Non AM + $CdCl_2$ (**C**), AM + $CdCl_2$ (**D**), Non AM + $ZnSO_4$ (**E**), and AM + $ZnSO_4$ (**F**)

Discussion

Selection of stress imparting concentrations of Cd and Zn

In the present study, 1.95 g Zn Kg^{-1} soil and 0.45 g Cd Kg^{-1} soil were selected as stress imparting concentrations because these concentrations caused ~50% growth reduction in *Z. mays* on 8 d of exposure. In the case of Zn, it was earlier proved by our own group that 1.95 g Zn Kg^{-1} soil caused ~50% growth reduction in *Z. mays* (Janeeshma et al. 2020) The 50% growth reduction was determined based on the enhancement in the MDA content and reduction in the total chlorophyll and tissue moisture content. But, the metal concentrations selected as stress imparting concentrations to plants, established in the previous reports were different from this. For a pot experiment conducted in three-month-old rice plants, the minimal inhibitory concentration of Cd was 3500 µg/ml (Mitra et al. 2018). Whereas the concentration of Cd

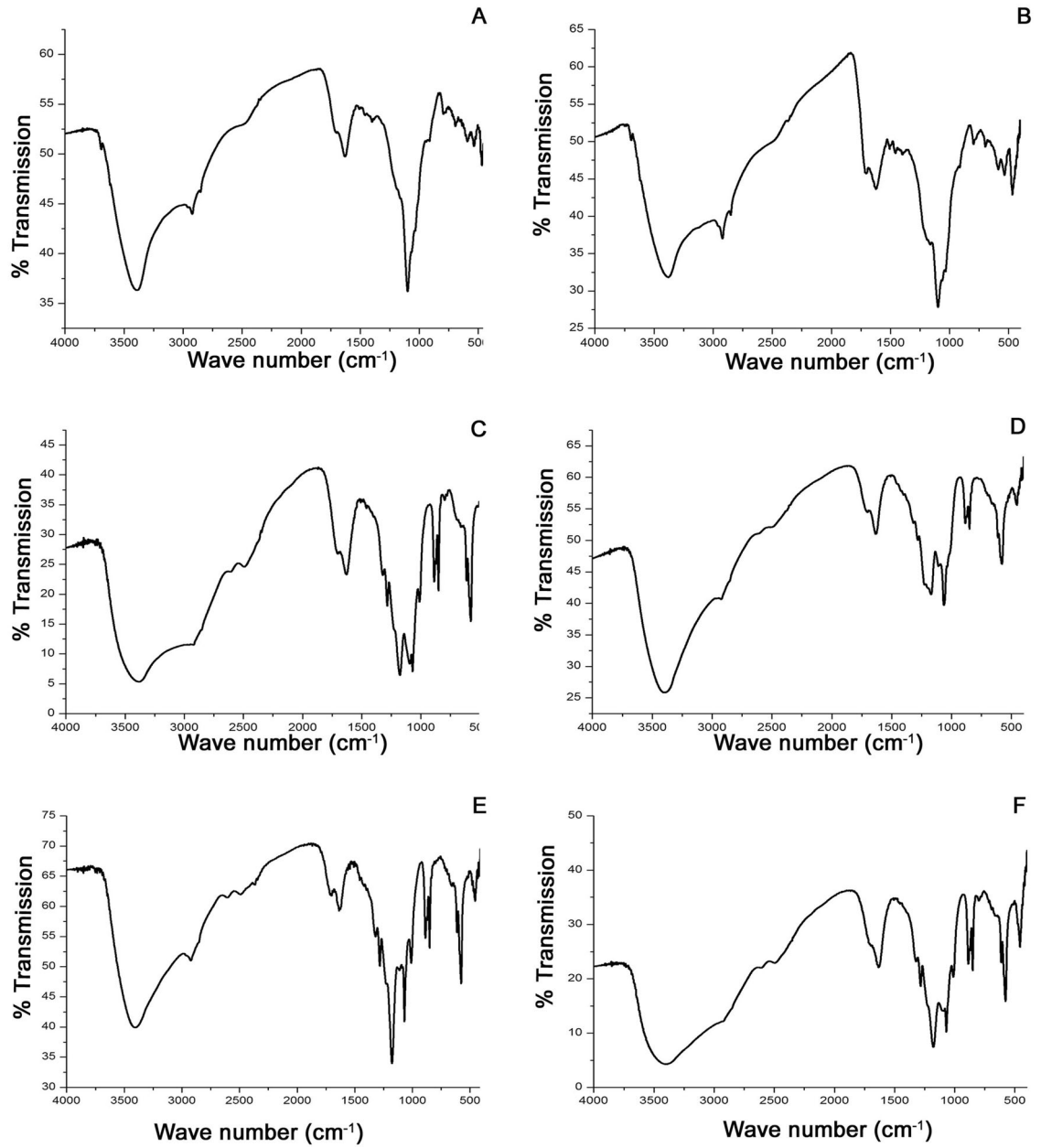

Fig. 6 Characterization of lignin isolated from the maize roots associated with mycorrhiza exposed to $ZnSO_4$ and $CdCl_2$; FTIR spectrum of Non AM (**A**), AM (**B**), Non AM + $CdCl_2$ (**C**), AM + $CdCl_2$ (**D**), Non AM + $ZnSO_4$ (**E**), and AM + $ZnSO_4$ (**F**)

selected for the experiments in *Brassica napus* was 15 µg Cd^{2+} g^{-1} soil (Dell'Amico et al. 2008). In the case of Zn, 300 µM $ZnSO_4$ solution caused toxic effects in *Beta vulgaris* in a hydroponics study. But in soil, 200 mg/kg of Zn caused growth retardation in 105 days old *Lycopersicon esculentum* (Vijayarengan and Mahalakshmi 2013). These variations in the stress imparting concentration of Cd and Zn depended on the duration of the study, soil type, plant species, etc. (Janeeshma and Puthur 2020).

Growth parameters

Mycorrhization induced an increase in plant growth of *Z. mays* as compared to plants without AM association. Earlier studies have reported mycorrhizal mediated enhancement in the shoot fresh weight and dry weight of *Z. mays* plants (Kothari et al. 1991; Danneberg et al. 1993; Zhao et al. 2015). This growth enhancement observed in AM plants was due to efficient phosphorous absorption and enhanced photosynthesis rate (Yang et al. 2021; Wu et al. 2020).

Table 5 Bioaccumulation of Cd and Zn (in mg g^{-1} DW) in maize plants associated with mycorrhiza exposed to $ZnSO_4$ and $CdCl_2$

Samples		0 d			4 d			8 d			12 d		
		Root	Shoot	TF	Root	Shoot	TF	Root	Shoot	TF	Root	Shoot	TF
Non AM	Zn	0.0459 ± 0.0023^{d}	0.0162 ± 0.0008^{b}	0.35	0.0541 ± 0.0027^{c}	0.0172 ± 0.0009^{c}	0.32	0.0541 ± 0.0027^{c}	0.0162 ± 0.0008^{d}	0.30	0.0541 ± 0.0027^{d}	0.0152 ± 0.0008^{d}	0.28
AM		0.0614 ± 0.0031^{b}	0.0274 ± 0.0014^{a}	0.45	0.0703 ± 0.0035^{c}	0.0261 ± 0.0013^{c}	0.37	0.0703 ± 0.0035^{c}	0.0281 ± 0.0014^{c}	0.40	0.0703 ± 0.0035^{c}	0.0251 ± 0.0013^{c}	0.36
Non AM + Zn		0.0541 ± 0.0027^{c}	0.0152 ± 0.0008^{b}	0.28	0.4417 ± 0.0221^{a}	0.1558 ± 0.0078^{a}	0.35	0.7409 ± 0.037^{a}	0.1222 ± 0.0061^{a}	0.16	0.8093 ± 0.0405^{a}	0.2984 ± 0.0149^{a}	0.37
AM + Zn		0.0703 ± 0.0035^{a}	0.0281 ± 0.0014^{a}	0.40	0.2592 ± 0.013^{b}	0.1127 ± 0.0056^{b}	0.43	0.251 ± 0.0013^{b}	0.0888 ± 0.0044^{b}	0.35	0.3588 ± 0.0179^{b}	0.1942 ± 0.0097^{b}	0.54
Non AM	Cd	BDL	BDL		BDL	BDL		BDL	BDL		BDL	BDL	
AM		BDL	BDL		BDL	BDL		BDL	BDL		BDL	BDL	
Non AM + Cd		BDL	BDL		0.6126 ± 0.0306^{b}	0.4326 ± 0.0216^{a}	0.71	5.0488 ± 0.2524^{a}	0.6094 ± 0.0305^{a}	0.12	3.3381 ± 0.1669^{a}	0.515 ± 0.0257^{b}	0.15
AM + Cd		BDL	BDL		0.7709 ± 0.0385^{a}	0.2178 ± 0.0109^{b}	0.28	3.7446 ± 0.1872^{b}	0.6525 ± 0.0326^{a}	0.17	2.6406 ± 0.132^{b}	0.6389 ± 0.0319^{a}	0.24

Values are expressed as mean ± SE of three independent experiments. Different letters superscript to the values indicate a significant difference between treatments (Duncan's test, $p \leq 0.05$). Where BDL indicates below detectable level

At the same time, Cd and Zn-induced reductions in the fresh weight of *Z. mays* were reported in various studies (El Dakak and Hassan 2020; Ahmad et al. 2019). Heavy metals significantly reduce water and mineral transport and impair the normal growth and development of the plants (Kandziora-Ciupa et al. 2017; Jimoh and Afolayan 2020; Liu et al. 2021). Moisture content in the leaf tissues of *Z. mays* was reduced due to the stress imparted by Cd and Zn. An appreciable reduction in the moisture content was observed in *Z. mays* due to Zn toxicity, where the moisture content showed a progressive reduction with an increase in the concentration of Zn (Janeeshma et al. 2020). This is due to the blockage of xylem water transport due to metal complexation (Sruthi and Puthur 2019). In contrast, the moisture content in the roots of *Z. mays* was increased under severe stress due to less water transport to the shoots, ending up in degeneration of root cells due to hyper-hydricity. *Solanum lycopersicum* exhibited a similar trend in root water status, where the root water content was increased in plants exposed to 10 d of Cd stress (300 µM of $CdCl_2$) (Zoghlami et al. 2011). In the present study, it was clear that mycorrhization aid to alleviate the reduction in the moisture content of the shoot and also prevents the excess of water storage in root cells. This may be due to the potential of mycorrhizae to maintain the transpiration rate similar to control under suboptimal conditions (Miransari 2010).

Primary metabolites

The drastic increase of sugar content observed in the leaves of maize plants under Cd and Zn stress indicates the upregulation of sugar metabolism so as to meet the higher carbon/energy requirement to cope up with the stress conditions (Rosa et al. 2009; Mishra et al. 2014). But such a trend may not remain the same, with an increase in the metal concentration, the sugar content decreases, denoting hindrance in the synthesis process of the same. Moreover, the senescence stage due to intolerable levels of metals could be the added reason for this. At a higher concentration of Cd (300 µM), the soluble sugars such as sucrose and glucose were reduced in the leaves and roots of *Solanum lycopersicum*, which indicates the synthesis process of these sugars is seriously hampered (Zoghlami et al. 2011). This phenomenon becomes more prominent in non AM plants, wherein the soluble sugar content showed a drastic reduction on 12 d of Cd and Zn exposure. The mycorrhizal association helped the plants to continue with the increased rate of sugar synthesis even on the 12 d of stress exposure. Similar results were obtained in *Z. mays* associated with *Glomus* sp. on exposure to Cd stress (Kumar and Dwivedi 2018). It indicates that mycorrhizae can maintain the biosynthesis of sugars in plants under metal stress, and it may be accomplished due to the efficient restriction in the uptake

and mobilization of Cd and Zn to the leaves. A previous study conducted in maize plants proved the mycorrhizal mediated stress mitigation with a special emphasis on the crucial role of soluble sugars (Mirshad and Puthur 2016; Kumar and Dwivedi 2018). Similar to our results, in the previous study conducted in maize plats, the mycorrhization increased the sugar content, and it potentially aids in overcoming the oxidative stress exerted by excess Cd ions (Kumar and Dwivedi 2018).

In the case of soluble protein, an increase was observed in the non AM and AM plants indicating the upregulation in the biosynthesis of enzymatic antioxidants and the stress-induced proteins (Shah et al. 2020; Labidi et al. 2021). These proteins are essential to mitigate the oxidative burst elicited in the leaves due to the uptake of the toxic heavy metals (Shackira and Puthur 2017). AM induced enhancement in the activity of different antioxidant enzymes involved in the ROS scavenging was observed in *Canavalia ensiformis* under elevated copper stress (Andrade et al. 2010). However, both non AM and AM plants showed a reduction in the protein synthesis on 12 d of Cd stress exposure, indicating the failure of these plants to continue with the same efficiency of protein biosynthesis at extreme stress. But in the case of *Z. mays* exposed to Zn stress, the protein biosynthesis increased on 12 d of stress showing that Zn had no major inhibitory action in the protein biosynthesis process of *Z. mays*. Cd (300 µM) stress caused a reduction in the soluble protein content in the leaves and roots of non AM *Solanum lycopersicum*, indicating the inhibition of the protein synthesis process as similar as to the senescence stage of the plants (Zoghlami et al. 2011). When maize plants with the mycorrhizal association were exposed to Zn stress, the degradation of proteins was prevented, and similar results were obtained in *Solanum lycopersicum* (Zoghlami et al. 2011). This may be due to the reduced toxic metal ions in AM plants that were observed in the results of the AAS analysis.

Accumulation of proline was observed in leaves of different plants in response to heavy metal stress, and this could be due to reduction in proline degradation, and/or increase in proline biosynthesis and also by hydrolysis of protein to free amino acids (Mirshad and Puthur 2016; Sameena and Puthur 2020). In the present study, the proline content of maize leaves increased in non AM and AM plants on exposure to Cd and Zn stressors. Non AM plants accumulated more proline as compared to AM plants and could be a strategy to counter the high level of Cd and Zn ions bioaccumulated in the shoot. Proline can effectively counter the metal stress in several ways; by mitigating metal-induced water deficit (Schat et al. 1997), protecting the activity of glucose-6-phosphate dehydrogenase and nitrate reductase (Sharma et al. 1998), and chelating the metal ions (Sharma et al. 1998). When *Cassia italica* was exposed to Cd stress, non AM plants accumulated more proline as compared to AM plants (Hashem et al. 2016a) which were similar to our observations. Contradictory to the above, it was seen that the proline content in roots was dramatically reduced in the roots of non AM plants exposed to Cd and Zn stress. But, mycorrhization lowered the reduction in the proline content under Cd as well as Zn toxicity, which enhanced the metal tolerance potential of maize plants. Association with AM fungi has been reported to induce proline synthesis in crop plants such as *Helianthus annuus, Solanum lycopersicum* and *Cajanus cajan* (Ef et al. 2015; Hashem et al. 2016b; Garg and Singh 2018). Mycorrhizae-induced proline accumulation helped to maintain high proline levels in the cells even under Cd and Zn stress. The negative correlation observed between proline and sugar content in non AM plants indicates that one does affect the biosynthesis of the other. A similar response was observed in pea plants exposed to metal stress; the increasing concentration of Cd caused an increase in proline content with a simultaneous reduction in sugar content (Sager et al. 2020). This could be due to the utilization of sugar as the carbon source of proline biosynthesis (Sager et al. 2020). However, AM plants accumulated proline and sugar simultaneously that aid to cope up with the extreme metal toxicity. AM plants exposed to different heavy metals exhibited a positive correlation between the accumulations of these two compounds; accumulation of proline could induce enhanced biosynthesis of sugars, as seen in the case of sugar beet (Ghaffari et al. 2021). The simultaneous enhancement of sugar and proline content in AM plants indicates the presence of a saturated carbon pool even under extreme metal stress, and thus for proline biosynthesis, the accumulated sugar was not utilized in these plants (Gurrieri et al. 2020; Ghaffari et al. 2021).

Accumulation of amino acids under heavy metal stress is a common strategy observed in different plants (Sharma and Dietz 2006; Sruthi and Puthur 2019). In this study, the leaves of non AM plants showed a drastic increase in the amino acids content at the early phase of metal exposure as compared to AM plants. When *Canavalia ensiformis* was exposed to copper stress, non AM plants showed an increase in the amino acids content than AM plants, and this is in line with our observations (Andrade et al. 2010). But at later stages, the amino acids content was drastically reduced in the non AM plants indicating Cd and Zn induced impairment in the amino acid biosynthesis. At the same time, mycorrhization elevated the accumulation of amino acids, indicating the capacity of AM plants to proceed with normal biosynthesis of amino acids even in extreme metal toxicity. Similar results were observed in *Cajanus cajan* associated with *Glomus mosseae* exposed to Cd stress, where free amino acid accumulation was increased due to the plant-fungus-metal interaction

(Garg and Chandel 2012). The reduction of metal transport observed in our results was the reason for the better amino acid biosynthesis of AM plants.

Secondary metabolites

Secondary metabolism changes dramatically in the leaves of mycorrhizal plants due to the hormonal variation induced by AM association (Copetta et al. 2006; Toussaint et al. 2007). Of the different secondary metabolites, phenolics compounds showed the greatest changes due to mycorrhization and heavy metal stresses (Kısa et al. 2016; Zhao et al. 2016). In this study, Cd and Zn-induced accumulation of phenolics compounds were observed in the leaves on 4 d of metal exposure, which points towards the immediate role of this secondary metabolite in ROS scavenging. The role of phenolics in ROS management was studied in *Withania somnifera* and *Vaccinium corymbosum*, and crucial contributions of phenolics in ROS scavenging were evidenced in both these studies (Manquián-Cerda et al. 2016; Mishra and Sangwan 2019). But on 12 d of stress exposure, the phenolics content was higher in AM plants, indicating a greater and prolonged role played by phenolics compounds in the metal tolerance of AM associated plants. Phenolics biosynthesis pathway proceeded uninterrupted in AM plants but not in non AM plants at extreme metal toxicity. In roots, generally the phenolics content decreases with exposure to metal stress, as observed in the case of *Linum usitatissimum* roots exposed to a high concentration of boron and aluminium (Heidarabadi et al. 2011). In the present study, reduction in the phenolics content was prominent in the roots of non AM plants as compared to AM plants, which indicates the potential of mycorrhization to mitigate the negative impact induced by HMs on the biosynthesis of phenolics.

Flavonoids are phenolic compounds with dihydroxy B-ring in their structure and can scavenge the ROS generated under heavy metal stress (Davies et al. 2018). Heavy metal-induced accumulation of flavonoids was reported in *Robinia pseudoacacia* (Zhao et al. 2016). An increase in the flavonoids content under Cd and Zn stress is a strategy to detoxify ROS molecules in the leaves of *Z. mays*. At earlier stages of the stress, mycorrhization insignificantly contributes to the flavonoid synthesis, but at the later stages of the stress, the mycorrhization aid to improve the flavonoids content, which supports the effective scavenging of ROS molecules. Anthocyanin is a water-soluble pigment with strong antioxidant and metal chelating properties, which showed an increase under metal toxicity (Landi et al. 2014; Janeeshma et al. 2020). Zn stress induced a drastic increase in the anthocyanin accumulation in the leaves of non AM and AM plants as compared to Cd stress. Therefore, it can be assumed that the intensity of anthocyanin accumulation is more specific to the metal Zn and the complexation of Zn with anthocyanin helps to maintain the metabolic process of the shoot (Janeeshma et al. 2020). Roots also showed an increase in anthocyanin accumulation, which aid in chelating the metal ions and transform metal ions into inactive forms. This result could correlate with the overexpressing of anthocyanin biosynthesizing genes under heavy metal stress that aid to immobilize more metal ions and subsequently increase the metal tolerance of plants (Ai et al. 2018; Gao et al. 2020).

Alkaloids are cyclic and nitrogen-containing compounds in plants that get elicited by different environmental stimuli. On exposure to lower concentrations of Cd and Pb, alkaloid compounds increased in the leaves of *Robinia pseudoacacia* (Zhao et al. 2016). Accordingly, both non AM and AM plants showed increased alkaloid content under Cd and Zn stresses, especially at the earlier days of the treatment period. But, with an increase in the days of exposure, the alkaloid content was decreased in maize plants. Similarly, a high concentration of Ni-induced inhibition in alkaloid production was reported in *Catharanthus roseus* (Idrees et al. 2013). At the same time, mycorrhization can further elicit alkaloid production in plants (Copetta et al. 2006; Toussaint et al. 2007). But, in the present study, the mycorrhizal association did not significantly contribute towards the alkaloid production, and thus both non AM and AM plants showed a reduction in the alkaloid content on 12 d of Cd and Zn stresses.

Bioactive compounds

In the present study, both qualitative and quantitative difference was observed in the phytochemical composition of both non AM and AM maize leaves. Mycorrhization potentially induces the biosynthesis of bioactive compounds, especially essential oils (Weisany et al. 2016). At the same time, heavy metal toxicity also elicits alterations in the composition of different bioactive compounds (Sruthi and Puthur 2019). In the present study, mycorrhization elicited the production of β-linalool and γ-sitosterol in the leaves of plants subjected to Cd stress. The accumulation of sterols is very important to plants, and the interaction of sterols with phospholipids helps plant cells to maintain plasma membrane fluidity and permeability during stress conditions (Aboobucker and Suza 2019). The accumulation of 3β-acetoxystigmasta-4, 6, 22-triene, which can activate the plasma membrane H^+-ATPase in non AM plants, aids in the transportation of protons out of the cells (Aboobucker and Suza 2019). The absence of this secondary metabolite in mycorrhizal plants indicates no ionic imbalance in AM plants due to the metal uptake as seen in non AM plants. The reduced metal translocation observed in AM plants was an aid to balance the secondary metabolites accumulation.

Neophytadiene is a diterpene, which acts as an anti-inflammatory agent, an antimicrobial agent and a plant metabolite. A reduction in the concentration of neophytadiene and an increase in the concentration of sterols under Cd treatment was observed in both AM and non AM plants, indicating Cd-induced inhibition in the biosynthesis of neophytadiene. However, at the same time, Cd stress induces the biosynthesis of sterols. These sterols have a crucial role in maintaining the plasma membrane fluidity and permeability by interacting with the phospholipids and help the plants to overcome the stress (Bartram et al. 2006; Aboobucker and Suza 2019). Even under the high concentrations of Zn, non AM and AM plants of maize did not exhibit any drastic changes in the phytochemistry, which indicates the higher tolerance of maize toward Zn toxicity than Cd stress.

The side chain of the chlorophyll molecule is known as phytol and gets released during the hydrolysis of this pigment. Moreover, it is a diterpene with antimicrobial, antioxidant, and anticancer activities. Stress-induced conversion of phytol into fatty acid phytol esters was reported by Lippold et al. (2012). The higher Rt value of the phytol (Rt-31.849) was due to the incorporation of lengthy hydrocarbon chains, and the resulting compound detected as (E)-Phytol. This change in the hydrocarbon chain can modify the normal ratio between (E)-Phytol and phytol in plants (Lippold et al. 2012). In the present study, Cd and Zn stressors altered the ratio between phytol and (E)-phytol in both mycorrhizal and non-mycorrhizal plants, and the mycorrhizal association did not have any specific role in maintaining the ratio between phytol and (E)-phytol in plants exposed to both Cd and Zn toxicity.

Lignin characterization

Lignin is made of three phenyl propane units of *p*-hydroxyphenyl, guaiacyl, and syringyl, with a variety of functional groups, including hydroxyls (phenolic and aliphatic hydroxyls), methoxyls, carbonyls, and carboxyls (Zhou et al. 2015). Lignin's most characteristic functional group is the hydroxyl groups (O-H), having the property of high reactivity (Santos et al. 2015). A wide peak at 3403 cm^{-1} corresponds to the aromatic and aliphatic OH groups, which is evident in all the lignin samples isolated from the shoot and roots of maize plants. Among the different hydroxyl groups, the phenolic hydroxyl group determines the lignin content increase as it aid in the progress of the polymerization reaction. Reduction of the peak at 3403 cm^{-1} in the shoots of Cd and Zn treated non AM and AM plants indicates a lower content of hydroxyl group, denoting that Cd and Zn stressors significantly hindered the polymerization of lignin chain (Thielemans and Wool 2005; Katahira et al. 2018).

The peaks at 1285, 1233, 1178 cm^{-1} correspond to the stretching of C-O groups; further, these peaks represent aromatic esters, alkyl or aryl ether and esters respectively. These three bands were prominently observed in the plants under both Cd and Zn stresses as compared to control plants, indicating the stress-induced esterification of lignin. Metal-induced esterification of lignin's hydroxyl groups can improve its organic solubility and hydrophobicity (Thielemans and Wool 2005). This hydroxyl esterification leads to decreased hydrogen bonding, reducing the permeability of metal ions through water uptake and thus lowers the metal uptake, protecting the plant from excess metal accumulation (Steudle 2000). Here, also mycorrhization did not significantly contribute towards the alterations in lignin composition and modifications.

The peaks at 2911, 2846, 1716, 1322, 1067, and 849 cm^{-1} are assigned to the symmetrical C–H stretching vibrations, asymmetrical C–H stretching vibration, stretching vibrations of C=O bonds in either ester linkages or carboxyl groups, syringil ring breathing with C-O stretching, C–H bending, and C-H groups respectively (Thielemans and Wool 2005). The peak at 616 cm^{-1} indicated sulphuric acid, which remains as a contaminant in the isolation protocol of lignin samples, where the peak corresponds to C–S stretching. These peaks were common to all the lignin samples isolated from the non mycorrhizal and mycorrhizal plants exposed to Cd and Zn stressors. Also, it could be deduced that metal toxicity and the mycorrhizal association did not elicit any changes in these specific functional groups of lignin.

Bioaccumulation of Cd and Zn

Mycorrhizal mediated reduction in the Cd accumulation of maize root was regarded as an important tolerance mechanism towards metal stress, and similar results were reported in *T. aestivum* by Shahabivand et al. (2012) and Sharma et al. (2016). Stabilization of metal ions and prevention of its uptake are common strategies of mycorrhizae to protect its symbionts from metal toxicity. With mycorrhizal association, different plants such as pigeonpea, rice, and maize plants showed similar tolerance mechanisms towards Cd toxicity (Liu et al. 2014; Garg et al. 2015; Rizwan et al. 2018).

In the present study, mycorrhization reduces the uptake of Zn ions to the roots, and the subsequent translocation of Zn into the maize leaves was also reduced as compared to non AM plants. The reduction in the uptake of Zn due to mycorrhization was reported by different researchers (Burleigh et al. 2003; Andrejić et al. 2018). Glomalin, a protein produced by the external mycelium of *Glomus*, can chelate metal ions (Gonzalez-Chavez et al. 2004). Moreover, the mycorrhizal hyphae also have a very important role in phytostabilization by enhancing the passive adsorption of

heavy metal to the hyphae, which aid in reducing the uptake of Zn ions by the root cells (Leyval and Joner 2001).

Conclusion

Dynamism observed in the quantity of primary and secondary metabolites such as soluble sugar, soluble protein, amino acid, proline, phenolics, alkaloids, flavonoids and anthocyanin content in maize plants under heavy metal stress signifies the importance of mycorrhization to reduce the impact of metal toxicity by altering the level of these metabolites. Metal-induced esterification of lignin's hydroxyl groups is another strategy of the plant to tolerate Cd and Zn toxicity. Mycorrhization did not contribute to any significant structural variations in lignin. The HCA of essential oils showed that the metal-induced metabolic alterations were prominent in the shoot. Mycorrhizae modified the root metabolites, which further helped to stabilize the metal ions in the root or rhizosphere and thus prevents the translocation of metals to shoots. The significant metabolic alterations in the root and stabilization of metal ions in the rhizosphere by the mycorrhizae potentially help the host plant to survive the Zn and Cd stresses. This research highlights several new mechanisms related to the role of arbuscular mycorrhizae fungus in ecological studies, such as contaminated soil recovery projects, which are based on the phytoremediation process.

Availability of data and materials

Data sharing is not applicable to this article as all new created data is already contained within this article.

Acknowledgements The authors greatly acknowledge the Centre for Plant Breeding and 24 Genetics, Department of Millets, Tamil Nadu Agriculture University (TNAU), Coimbatore, 25 India, for providing seeds essential to conduct the experiments and Centre for Mycorrhizal 26 Culture Collection (CMCC), The Energy and Resources Institute (TERI), New Delhi for 27 providing the inoculum of mycorrhiza. The authors extend their sincere thanks to the Department 28 of Science & Technology (DST), Government of India for granting funds under the Fund for 29 Improvement of S&T Infrastructure (FIST) programme (SR/FST/LSI-532/2012).

Author contributions EJ performed the analysis, processed the experimental data, interpreted the results, drafted the paper and designed the Figures. JTP provided critical feedback and helped shape the research and analysis aided in interpreting the results, and worked on the paper. HMK gave critical input and helped develop the study and interpretation that helped to understand the findings and work on the paper. JW offered valuable insight and helped to establish the research and analysis that helped to explain the paper observations and function.

Funding This work was supported by the University Grant Commission (UGC), India in the form of JRF under grant 319492.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

Aboobucker SI, Suza WP (2019) Why do plants convert sitosterol to stigmasterol? Front Plant Sci 10:354. https://doi.org/10.3389/fpls.2019.00354

Adeyemi NO, Atayese MO, Sakariyawo OS, Azeez JO, Sobowale SPA, Olubode A, Adeoye S (2021) Alleviation of heavy metal stress by arbuscular mycorrhizal symbiosis in *Glycine max* (L.) grown in copper, lead and zinc contaminated soils. Rhizosphere 18:100325. https://doi.org/10.1016/j.rhisph.2021.100325

Ahmad I, Zaib S, Alves PCMS, Luthe DS, Bano A, Shakeel SN (2019) Molecular and physiological analysis of drought stress responses in *Zea mays* treated with plant growth promoting rhizobacteria. Biol Plant 63:536–547. https://doi.org/10.32615/bp.2019.092

Ai TN, Naing AH, Yun BW, Lim SH, Kim CK (2018) Overexpression of RsMYB1 enhances anthocyanin accumulation and heavy metal stress tolerance in transgenic petunia. Front Plant Sci 9:1388. https://doi.org/10.3389/fpls.2018.01388

Allan JE (1969) The preparation of agricultural samples for analysis by Atomic Absorption Spectrometry. Varian Techtron Bulletin SIS Edn 12:69

Andrade SA, Gratão PL, Azevedo RA, Silveira AP, Schiavinato MA, Mazzafera P (2010) Biochemical and physiological changes in jack bean under mycorrhizal symbiosis growing in soil with increasing Cu concentrations. Environ Exp Bot 68:198–207. https://doi.org/10.1016/j.envexpbot.2009.11.009

Andrejić G, Gajić G, Prica M, Dželetović Ž, Rakić T (2018) Zinc accumulation, photosynthetic gas exchange, and chlorophyll a fluorescence in Zn-stressed *Miscanthus*× *giganteus* plants. Photosynthetica 56:1249–1258. https://doi.org/10.1007/s11099-018-0827-3

Bartram S, Jux A, Gleixner G, Boland W (2006) Dynamic pathway allocation in early terpenoid biosynthesis of stress-induced lima bean leaves. Phytochemistry 67:1661–1672. https://doi.org/10.1016/j.phytochem.2006.02.004

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207. https://doi.org/10.1007/BF00018060

Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ashraf M et al. (2019) Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. Front Plant Sci 10:1068

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein dye binding. Anal Biochem 72:248–54

Burleigh SH, Kristensen BK, Bechmann IE (2003) A plasma membrane zinc transporter from *Medicago truncatula* is up-regulated in roots by Zn fertilization, yet down-regulated by arbuscular mycorrhizal colonization. Plant Mol Biol 52:1077–1088. https://doi.org/10.1023/A:1025479701246

Chen JX, Wang XF (2006) Guidance for Plant Physiological Experiment. South China University of Technology Press, Guangzhou, China

Copetta A, Lingua G, Berta G (2006) Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum* L. var. Genovese. Mycorrhiza 16:485–494. https://doi.org/10.1007/s00572-006-0065

Danneberg G, Latus C, Zimmer W, Hundeshagen B, Bothe H (1993) Influence of vesicular-arbuscular mycorrhiza on phytohormone balances in maize (*Zea mays* L.). J Plant Physiol 141:33–39

Davies KM, Albert NW, Zhou Y, Schwinn KE (2018) Functions of flavonoid and βlain pigments in abiotic stress tolerance in plants. Annu Rev Plant Biol 41:21–62. https://doi.org/10.1002/9781119312994.apr0604

Dell'Amico E, Cavalca L, Andreoni V (2008) Improvement of *Brassica napus* growth under cadmium stress by cadmium-resistant rhizobacteria. Soil Biol Biochem 40:74–84. https://doi.org/10.1016/j.soilbio.2007.06.024

Domínguez-Robles J, Sánchez R, Espinosa E, Savy D, Mazzei P, Piccolo A, Rodríguez A (2017) Isolation and characterization of gramineae and fabaceae soda lignins. Int J Mol Sci 18:327. https://doi.org/10.3390/ijms18020327

Dubois M, Gilles KA, Hamilton JK, Rebers PT, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356. https://doi.org/10.1021/ac60111a017

Ef A, Abeer H, Alqarawi AA, Hend AA (2015) Alleviation of adverse impact of cadmium stress in sunflower (*Helianthus annuus* L.) by arbuscular mycorrhizal fungi. Pak J Bot 47:785–795

El Dakak RA, Hassan IA (2020) The alleviative effects of salicylic acid on physiological indices and defense mechanisms of maize (*Zea Mays* L. Giza 2) stressed with cadmium. Environ Processes 7:873–884

Folin O, Denis W (1915) A colorimetric method for the determination of phenol (and derivatives) in urine. J Biol Chem 22:305–308. https://doi.org/10.1016/S0021-9258(18)87648-7

Garg N, Chandel S (2012) Role of arbuscular mycorrhizal (AM) fungi on growth, cadmium uptake, osmolyte, and phytochelatin synthesis in *Cajanus cajan* (L.) Millsp. under NaCl and Cd stresses. J Plant Growth Regul 31:292–308. https://doi.org/10.1007/s00344-011-9239-3

Garg N, Singh S (2018) Arbuscular mycorrhiza *Rhizophagus irregularis* and silicon modulate growth, proline biosynthesis and yield in *Cajanus cajan* L. Millsp. (pigeon pea) genotypes under cadmium and zinc stress. J Plant Growth Regul 37:46–63. https://doi.org/10.1007/s00344-017-9708-4

Garg N, Singla P, Bhandari P (2015) Metal uptake, oxidative metabolism, and mycorrhization in pigeon pea and pea under arsenic and cadmium stress. Turk J Agric For 39:234–250

Gao J, Chen B, Lin H, Liu Y, Wei Y, Chen F, Li W (2020) Identification and characterization of the glutathione S-transferase (GST) family in radish reveals a likely role in anthocyanin biosynthesis and heavy metal stress tolerance. Gene 743:144484. https://doi.org/10.3389/fpls.2018.01388

Ghaffari H, Tadayon MR, Bahador M, Razmjoo J (2021) Investigation of the proline role in controlling traits related to sugar and root yield of sugar beet under water deficit conditions. Agric Water Manag 243:106448. https://doi.org/10.1016/j.agwat.2020.106448

Gonzalez-Chavez MC, Carrillo-Gonzalez R, Wright SF, Nichols KA (2004) The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environ Pollut 130:317–323. https://doi.org/10.1016/j.envpol.2004.01.004

Grover N, Patni V (2013) Phytochemical characterization using various solvent extracts and GC-MS analysis of methanolic extract of *Woodfordia fruticosa* (L) Kurz. Leaves. Int J Pharm Sci 5:91–5

Gurrieri L, Merico M, Trost P, Forlani G, Sparla F (2020) Impact of drought on soluble sugars and free proline content in selected *Arabidopsis* mutants. Biology 9:367. https://doi.org/10.3390/biology9110367

Hashem A, Abd-Allah EF, Alqarawi AA, Al Huqail AA, Egamberdieva D, Wirth S (2016b) Alleviation of cadmium stress in *Solanum lycopersicum* L. by arbuscular mycorrhizal fungi via induction of acquired systemic tolerance. Saudi J Biol Sci 23:272–281. https://doi.org/10.1016/j.sjbs.2015.11.002

Hashem A, Abd-Allah EF, Alqarawi AA, Egamberdieva D (2016a) Bioremediation of adverse impact of cadmium toxicity on *Cassia italica* Mill by arbuscular mycorrhizal fungi. Saudi J Biol Sci 23:39–47. https://doi.org/10.1016/j.sjbs.2015.11.007

Heidarabadi MD, Ghanati F, Fujiwara T (2011) Interaction between boron and aluminum and their effects on phenolic metabolism of *Linum usitatissimum* L. roots. Plant Physiol Biochem 49:1377–1383. https://doi.org/10.1016/j.plaphy.2011.09.008

Idrees M, Naeem M, Aftab T, Khan MMA (2013) Salicylic acid restrains nickel toxicity, improves antioxidant defence system and enhances the production of anticancer alkaloids in *Catharanthus roseus* (L.). J. Hazard Mater 252:367–374. https://doi.org/10.1016/j.jhazmat.2013.03.005.

Janeeshma E, Puthur JT (2020) Direct and indirect influence of arbuscular mycorrhizae on enhancing metal tolerance of plants. Arch Microbiol 202:1–16. https://doi.org/10.1007/s00203-019-01730-z

Janeeshma E, Rajan VK, Puthur (2020) Spectral variations associated with anthocyanin accumulation; an apt tool to evaluate zinc stress in *Zea mays* L. Chem Ecol 31:1–18

Jimoh MO, Afolayan AJ (2020) Heavy metal uptake and growth characteristics of *Amaranthus caudatus* L. under five different soils in a controlled environment. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 48:417–425

Kandziora-Ciupa M, Nadgórska-Socha A, Barczyk G, Ciepał R (2017) Bioaccumulation of heavy metals and ecophysiological responses to heavy metal stress in selected populations of *Vaccinium myrtillus* L. and *Vaccinium vitis-idaea* L. Ecotoxicology 26:966–980. https://doi.org/10.1007/s10646-017-1825-0

Katahira R, Elder TJ, Beckham GT (2018) A brief introduction to lignin structure. 1–20. https://doi.org/10.1039/9781788010351-00001

Kaya C, Ashraf M, Akram NA (2018) Hydrogen sulfide regulates the levels of key metabolites and antioxidant defense system to counteract oxidative stress in pepper (*Capsicum annuum* L.) plants exposed to high zinc regime. Environ Sci Pollut Res 25:12612–12618. https://doi.org/10.1007/s11356-018-1510-8

Kısa D, Elmastaş M, Öztürk L, Kayır Ö (2016) Responses of the phenolic compounds of *Zea mays* under heavy metal stress. Appl Biol Chem 59:813–820. https://doi.org/10.1007/s13765-016-0229-9

Kothari SK, Marschner H, Römheld V (1991) Contribution of the VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in a calcareous soil. Plant Soil 131:177–185

Kumar P, Dwivedi P (2018) Putrescine and *Glomus* mycorrhiza moderate cadmium actuated stress reactions in *Zea mays* L. by means of extraordinary reference to sugar and protein. Vegetos 31:74–77. https://doi.org/10.5958/2229-4473.2018.00076.9

Labidi O, Vives-Peris V, Gómez-Cadenas A, Pérez-Clemente RM, Sleimi N (2021) Assessing of growth, antioxidant enzymes, and phytohormone regulation in Cucurbita pepo under cadmium stress. Food Sci Nutr 9:2021–2031. https://doi.org/10.1002/fsn3.2169

Landi M, Guidi L, Pardossi A, Tattini M, Gould KS (2014) Photoprotection by foliar anthocyanins mitigates effects of boron toxicity in sweet basil (*Ocimum* basilicum). Planta 240:941–953. https://doi.org/10.1007/s00425-014-2087-1

Leyval C, Joner EJ (2001) Bioavailability of heavy metals in the mycorrhizosphere. In: Gobran GR, Wenze WW, Lombi E (Eds.) Trace elements in the rhizosphere. CRC Press, New York, NY, p 173–193

Lippold F, vom Dorp K, Abraham M, Hölzl G, Wewer V, Yilmaz JL et al. (2012) Fatty acid phytyl ester synthesis in chloroplasts of *Arabidopsis*. Plant Cell 24:2001–2014. https://doi.org/10.1105/tpc.112.095588

Liu L, Gong Z, Zhang Y, Li P (2014) Growth, cadmium uptake and accumulation of maize (*Zea mays* L.) under the effects of arbuscular mycorrhizal fungi. Ecotoxicology 23:1979–1986. https://doi.org/10.1007/s10646-014-1331-6

Liu Z, Bai Y, Luo L, Wan J, Wang W, Zhao G (2021) Effects of high dose copper on plant growth and mineral nutrient (Zn, Fe, Mg, K, Ca) uptake in spinach. Environ Sci Pollut Res 1:11. https://doi.org/10.1007/s11356-021-13395-7

Lokhande RS, Singare PU, Pimple DS (2011) Toxicity study of heavy metals pollutants in waste water effluent samples collected from Taloja industrial estate of Mumbai, India. Resources Environ 1:13–19. https://doi.org/10.5923/j.re.20110101.02

Mancinelli AL, Yang CPH, Lindquist P, Anderson OR, Rabino I (1975) Photocontrol of anthocyanin synthesis: III. The action of streptomycin on the synthesis of chlorophyll and anthocyanin. Plant Physiol 55:251–257. https://doi.org/10.1104/pp.55.2.251

Manquián-Cerda K, Escudey M, Zúñiga G, Arancibia-Miranda N, Molina M, Cruces E (2016) Effect of cadmium on phenolic compounds, antioxidant enzyme activity and oxidative stress in blueberry (*Vaccinium corymbosum* L.) plantlets grown in vitro. Ecotoxicol Environ Saf 133:316–326. https://doi.org/10.1016/j.ecoenv.2016.07.029

Mendoza-Soto AB, Naya L, Leija A, Hernández G (2015) Responses of symbiotic nitrogen-fixing common bean to aluminum toxicity and delineation of nodule responsive microRNAs. Front Plant Sci 6:587

Miransari M (2010) Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stress. Plant Biol 12:563–569

Mirecki RM, Teramura AH (1984) Effects of ultraviolet-B irradiance on soybean: V. The dependence of plant sensitivity on the photosynthetic photon flux density during and after leaf expansion. Plant Physiol 74:475–480. https://doi.org/10.1104/pp.74.3.475

Mirshad PP, Puthur JT (2016) Arbuscular mycorrhizal association enhances drought tolerance potential of promising bioenergy grass (*Saccharum arundinaceum* retz.). Environ Monit Assess 188:1–20. https://doi.org/10.1007/s10661-016-5428-7

Mishra B, Sangwan NS (2019) Amelioration of cadmium stress in *Withania somnifera* by ROS management: active participation of primary and secondary metabolism. Plant Growth Regul 87:403–412. https://doi.org/10.1007/s10725-019-00480-8

Mishra B, Sangwan RS, Mishra S, Jadaun JS, Sabir F, Sangwan NS (2014) Effect of cadmium stress on inductive enzymatic and nonenzymatic responses of ROS and sugar metabolism in multiple shoot cultures of Ashwagandha (*Withania somnifera* Dunal). Protoplasma 251:1031–1045. https://doi.org/10.1007/s00709-014-0613-4

Mitra S, Pramanik K, Sarkar A, Ghosh PK, Soren T, Maiti TK (2018) Bioaccumulation of cadmium by *Enterobacter* sp. and enhancement of rice seedling growth under cadmium stress. Ecotoxicol Environ Saf 156:183–196. https://doi.org/10.1016/j.ecoenv.2018.03.001

Moore S, Stein WH (1948) Photometric ninhydrin method for use in the chromatography of amino acids. J Biol Chem 176:367–388

Naz R, Sarfraz A, Anwar Z, Yasmin H, Nosheen A, Keyani R, Roberts TH (2021) Combined ability of salicylic acid and spermidine to mitigate the individual and interactive effects of drought and chromium stress in maize (*Zea mays* L.). Plant Physiol Biochem 159:285–300. https://doi.org/10.1016/j.plaphy.2020.12.022

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br Mycol Soc 55:158–161. https://doi.org/10.1016/S0007-1536(70)80110-3

Raj H, Sharma SD (2009) Integration of soil solarization and chemical sterilization with beneficial microorganisms for the control of white root rot and growth of nursery apple. Sci Hortic 119:126–131

Rivero J, Gamir J, Aroca R, Pozo MJ, Flors V (2015) Metabolic transition in mycorrhizal tomato roots. Front Microbiol 6:598. https://doi.org/10.3389/fmicb.2015.00598

Rizwan M, Ali S, Abbas T, Adrees M, Zia-ur-Rehman M, Ibrahim M et al. (2018) Residual effects of biochar on growth, photosynthesis and cadmium uptake in rice (*Oryza sativa* L.) under Cd stress with different water conditions. J Environ Manage 206:676–683. https://doi.org/10.1016/j.jenvman.2017.10.035

Rosa M, Prado C, Podazza G, Interdonato R, Juan A, Hilal M, Prado FE (2009) Soluble sugars: metabolism, sensing and abiotic stress. Plant Signal Behav 4:388–393

Rostami M, Mohammadi H, Müller T, Mirzaeitalarposhti R (2020) Silicon application affects cadmium translocation and physiological traits of *Lallemantia royleana* under cadmium stress. J Plant Nutr 43:753–761. https://doi.org/10.1080/01904167.2019.1701022

Sager SMA, Wijaya L, Alyemeni MN, Hatamleh AA, Ahmad P (2020) Impact of different cadmium concentrations on two *Pisum sativum* l. genotypes. Pak J Bot 52:821–829

Sameena PP, Puthur JT (2020) Cotyledonary leaves effectively shield the true leaves in *Ricinus communis* L. from copper toxicity. Int J Phytoremediation 10:1–13. https://doi.org/10.1080/15226514.2020.1825331

Santos JI, Martín-Sampedro R, Fillat Ú, Oliva JM, Negro MJ, Ballesteros M, Ibarra D (2015) Evaluating lignin-rich residues from biochemical ethanol production of wheat straw and olive tree pruning by FTIR and 2D-NMR. Int J Polym Sci 2015. https://doi.org/10.1155/2015/314891

Sarath NG, Puthur JT (2020) Heavy metal pollution assessment in a mangrove ecosystem scheduled as a community reserve. Wetlands Ecol Manage 11:1–12. https://doi.org/10.1007/s11273-020-09764-7

Schat H, Sharma SS, Vooijs R (1997) Heavy metal-induced accumulation of free proline in a metal-tolerant and a nontolerant ecotype of *Silene vulgaris*. Physiol Plant 101:477–482. https://doi.org/10.1111/j.1399-3054.1997.tb01026.x

Shackira AM, Puthur JT (2017) Enhanced phytostabilization of cadmium by a halophyte—Acanthus ilicifolius L. Int J Phytoremediation 19:319–326

Shah AA, Ahmed S, Ali A, Yasin NA (2020) 2-Hydroxymelatonin mitigates cadmium stress in *Cucumis sativus* seedlings: modulation of antioxidant enzymes and polyamines. Chemosphere 243:125308. https://doi.org/10.1016/j.chemosphere.2019.125308

Shahabivand S, Maivan HZ, Goltapeh EM, Sharifi M, Aliloo AA (2012) The effects of root endophyte and arbuscular mycorrhizal fungi on growth and cadmium accumulation in wheat under cadmium toxicity. Plant Physiol Biochem 60:53–58. https://doi.org/10.1016/j.plaphy.2012.07.018

Shahzad A, Qin M, Elahie M, Naeem M, Bashir T, Yasmin H et al. (2021) *Bacillus pumilus* induced tolerance of Maize (*Zea mays* L.) against Cadmium (Cd) stress. Sci Rep 11:1–11. https://doi.org/10.1038/s41598-021-96786-7

Sharma SS, Dietz KJ (2006) The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. J Exp Bot 57:711–726. https://doi.org/10.1093/jxb/erj073

Sharma SS, Schat H, Vooijs R (1998) In vitro alleviation of heavy metal-induced enzyme inhibition by proline. Phytochemistry 49:1531–1535. https://doi.org/10.1016/S0031-9422(98)00282-9

Sharma V, Parmar P, Kumari N (2016) Differential cadmium stress tolerance in wheat genotypes under mycorrhizal association. J Plant Nutr 39:2025–2036. https://doi.org/10.1080/01904167.2016.1170851

Sruthi P, Puthur JT (2019) Characterization of physiochemical and anatomical features associated with enhanced phytostabilization of copper in *Bruguiera cylindrica* (L.) Blume. Int J phytoremediation 21:1423–1441

Steudle E (2000) Water uptake by roots: effects of water deficit. J Exp Bot 51:1531–1542. https://doi.org/10.1093/jexbot/51.350.1531

Talluri MR, Ketha A, Battu GR, Tadi RS, Tatipamula VB (2018) Protective effect of Aurelia aurita against free radicals and streptozotocin-induced diabetes. Bangladesh J Pharmacol 13:287–295

Thielemans W, Wool RP (2005) Lignin esters for use in unsaturated thermosets: lignin modification and solubility modeling. Biomacromolecules 6:1895–1905. https://doi.org/10.1021/bm0500345

Toussaint JP, Smith FA, Smith SE (2007) Arbuscular mycorrhizal fungi can induce the production of phytochemicals in sweet basil irrespective of phosphorus nutrition. Mycorrhiza 17:291–297. https://doi.org/10.1007/s00572-006-0104-3

Tovar-Sánchez E, Cervantes-Ramírez T, Castañeda-Bautista J, Gómez-Arroyo S, Ortiz-Hernández L, Sánchez-Salinas E, Mussali-Galante P (2018) Response of *Zea mays* to multimetal contaminated soils: a multibiomarker approach. Ecotoxicology 27:1161–1177. https://doi.org/10.1007/s10646-018-1974-9

Vijayarengan P, Mahalakshmi G (2013) Zinc toxicity in tomato plants. World Appl Sci J 24:649–653

Weisany W, Raei Y, Salmasi SZ, Sohrabi Y, Ghassemi-Golezani K (2016) Arbuscular mycorrhizal fungi induced changes in rhizosphere, essential oil and mineral nutrients uptake in dill/common bean intercropping system. Ann Appl Biol 169:384–397. https://doi.org/10.1111/aab.12309

Wu JT, Wang L, Zhao L, Huang XC, Ma F (2020) Arbuscular mycorrhizal fungi effect growth and photosynthesis of *Phragmites australis* (Cav.) Trin ex. Steudel under copper stress. Plant Biol 22:62–69

Yang L, Zou YN, Tian ZH, Wu QS, Kuča K (2021) Effects of beneficial endophytic fungal inoculants on plant growth and nutrient absorption of trifoliate orange seedlings. Sci Hortic 277:109815

Zhao R, Guo W, Bi N, Guo J, Wang L, Zhao J, Zhang J (2015) Arbuscular mycorrhizal fungi affect the growth, nutrient uptake and water status of maize (*Zea mays* L.) grown in two types of coal mine spoils under drought stress. Appl Soil Ecol 88:41–49

Zhao YH, Jia X, Wang WK, Liu T, Huang SP, Yang MY (2016) Growth under elevated air temperature alters secondary metabolites in *Robinia pseudoacacia* L. seedlings in Cd-and Pb-contaminated soils. Sci Total Environ 565:586–594. https://doi.org/10.1016/j.scitotenv.2016.05.058

Zhou C, Jiang W, Via BK, Fasina O, Han G (2015) Prediction of mixed hardwood lignin and carbohydrate content using ATR-FTIR and FT-NIR. Carbohydr Polym 121:336–341. https://doi.org/10.1016/j.carbpol.2014.11.062

Zoghlami LB, Djebali W, Abbes Z, Hediji H, Maucourt M, Moing A et al. (2011) Metabolite modifications in *Solanum lycopersicum* roots and leaves under cadmium stress. Afr J Biotechnol 10:567–579