Heavy metal tolerance mechanism in *Zea mays* L. and *Oryza sativa* L. associated with arbuscular mycorrhiza

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By

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This is to certify that all the corrections mentioned by the adjudicators in the thesis entitled **"Heavy metal tolerance mechanism in *Zea mays* L. and *Oryza sativa* L. associated with arbuscular mycorrhiza"** submitted by **JANEESHMA E.,** have been incorporated as per the adjudication report. It is also certified that the contents in the thesis and the CD submitted are one and the same.

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DECLARATION

I, **Janeeshma E.,** do hereby declare that the Ph.D thesis entitled **"Heavy metal tolerance mechanism in *Zea mays* L. and *Oryza sativa* L. associated with arbuscular mycorrhiza"** is a research work accomplished by me under the supervision of **Dr. Jos, T. Puthur, Professor, Plant Physiology and Biochemistry Division, Department of Botany, University of Calicut,** in partial fulfillment of the requirements for the award of **Doctor of Philosophy** in **Botany, University of Calicut.** I also declare that this has not been submitted by me for the award of any other degree or diploma, and it represents original work done by me.

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Heavy metal tolerance mechanism in *Zea mays* L. and *Oryza sativa* L. associated with arbuscular mycorrhiza

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Abstract

The present study was carried out to investigate the modifications in the heavy metal stress tolerance potential of *Oryza sativa* (variety Varsha) and *Zea mays* (variety CoHM6) having association with mycorrhizae. Various analysis were conducted in the leaves and the roots of *O. sativa* and *Z. mays* to evaluate the functional aspects, metabolic implications and structural remodulations in the plants associated with mycorrhizae and exposed to Cd and Zn stresses. The extensive ramification of hyphae along with the development of arbuscules and vesicles of *Claroideoglomus claroideum* and *C. etunicatum* in the roots of *O. sativa* and *Z. mays* proved the wide spectrum of host specificity and compatibility of these microbes with the members of monocots. Heavy metal treatment induced inhibition of the growth, photosynthesis, alterations in the metabolomics, anatomical modifications in *O. sativa* and *Z. mays*. The present study evidenced the efficiency of mycorrhization to ameliorate the adverse effects of Cd and Zn stresses in *Z. mays* and *O. sativa*, by sequestering toxic metal ions in AM fungal structures, mitigating the oxidative stress, maintaining the metabolic status, boosting the antioxidant activity, and altering the bioaccumulation pattern of the metals in the hosts.

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ABBREVIATIONS

ABS	-	Absorption flux
AM	-	Arbuscular mycorrhiza
APX	-	Ascorbate peroxidase
AsA	-	Ascorbate
CAT	-	Catalase
Cd	-	Cadmium
CS_M	-	Cross section related to F_M
DCMU	-	3 (3,4dichlorophenyl) -1,1-dimethyl urea
DCPIP	-	2,6-dicholrophenolindophenol
DIo	-	Dissipated energy flux
DTNB	-	5-dithio-bis-2-nitrobenzoic acid
DW	-	Dry weight
EDTA	-	Ethylene diamine tetra acetic acid
EDXMA	-	Energy Dispersive X-ray Microanalysis
EL%	-	Electrolyte leakage
ETo	-	Electron transport flux
F_M	-	Maximum Chl *a* fluorescence
F_O	-	Initial Chl *a* fluorescence
FT-IR	-	Fourier Transform Infrared
F_V	-	Variable Chl *a* fluorescence
FW	-	Fresh weight
GC-MS	-	Gas Chromatography and Mass Spectrometry
GPOX	-	Guaiacol peroxidase
GSH	-	Reduced glutathione
GSSG	-	Oxidized glutathione
H_2O_2	-	Hydrogen peroxide
HEPES	-	[N-(2-Hydroxyethyl)piperazine-N-(2-Ethanesulphonic acid)
HM	-	Heavy metal
IBA	-	Indole-3-butyric acid
KBr	-	Potassium bromide
LHC	-	Light harvesting complex
MC%	-	Moisture content %

MD	-	Mycorrhizal dependency
MDA	-	Malondialdehyde
MSI	-	Membrane stability index
MT	-	Metallothionein
MV	-	Methyl viologen
NBT	-	Nitroblue tetrazolium
$O_2^{\cdot -}$	-	Superoxide
pBQ	-	Parabenzoquinone
PCs	-	Phytochelatins
PI	-	Performance index
PSI	-	Photosystem I
PSII	-	Photosystem II
RC	-	Reaction center
ROS	-	Reactive oxygen species
SEM	-	Scanning Electron Microscope
SOD	-	Superoxide dismutase
TBA	-	Thiobarbituric acid
TCA	-	Trichloroacetic acid
TF	-	Translocation factor
TI	-	Tolerance index
Zn	-	Zinc

INTRODUCTION

Environmental pollution is the end result of undesirable changes happening to the natural characteristics of air, water, soil and the biota due to the acceleration of urbanization and industrialization. Among the different pollutions, heavy metal (HM) toxicity is a growing concern for human population due to the long persistence, bioaccumulation and biomagnification properties of these xenobiotics (Yin et al., 2018). Since 19th century, due to different anthropogenic activities, the intensity of heavy metal pollution is increasing in the environment (Kumar et al., 2019). The global heavy metal pollution index (HPI) exceeded the limit (100 as per the guidelines given by U.S. Environmental Protection Agency) and reached to 3560 (Kumar et al., 2019). Agricultural lands are under a big threat of getting polluted with heavy metals and metalloids such as cadmium (Cd), zinc (Zn), arsenic (As), cobalt (Co), silver (Ag), gold (Au), nickel (Ni) copper (Cu) and lead (Pb) owing to the mismanaged agricultural practices, industrial waste disposal, smelting and mining (Anjum et al., 2015; Yang et al., 2018). Indian arable lands are also extensively contaminated with these heavy metals due to the continuous usage of contaminated waste water for irrigation (Dotaniya and Pipalde, 2018). On closely examining the status of the state Kerala, similar trend in heavy metal accumulation was observed in the different soil environment located at coastal lake (Akkulam–Veli) (Sheela et al., 2012), Cochin backwaters (Anu et al., 2014), and national highway-47 passing through Thiruvananthapuram district (Jaya, 2009).

Translocation of toxic metal ions to the grains of crop plants increases the possibility of hyperaccumulation of the same in humans, which leads to different toxicity symptoms like bone mineralization and cancer (Ma et al., 2015). Heavy metals also damages the normal functioning of vital organs in

the human body such as brain, lungs, kidney, liver and it also alters the blood composition. The intensity in which HM toxicity affects humans was imprinted in the history as 'minamata' disease and 'itai-itai' disesae. 'Minamata' is a neurological disease representing the chronic mercury toxicity (Japan, 1956) with the symptoms such as ataxia (a degenerative disease to nervous system resulting in impairment of coordination in muscle movements), lack of sensation in the hands and feet, general muscle weakness, narrow field of vision and damage to hearing and speech (Harada, 1995). In 1968, Japan faced similar event, caused by Cd toxicity which is known as 'itai-itai', with osteomalaecia (condition where bones become soft and weak) and associated renal tubular dysfunctions (Nishijo et al., 2017). Both the metals reached the human population by biomagnification through food chain. In addition to this, the high metal accumulation potential of *O. sativa* plants added to the impact of the 'itai-itai' disease, which implies the significant role of crop plants in the transfer of heavy metals from environment to the human population.

The crop plants are getting negatively affected due to the exposure to high concentrations of HMs. Among the different heavy metals, Cd and Zn are potentially toxic to plants and animals because of their increased bioavailability. Cadmium and Zn toxicity impairs the plant metabolisms like photosynthesis, respiration, transpiration, nitrogen metabolism and finally the normal growth and development of plants (Paunov et al., 2018). Moreover, Cd and Zn toxicity inhibited the root growth of *Zea mays* seedlings (Malekzadeh et al., 2007). Crop yield and quality were also reduced due to the phytotoxicity induced by Cd and Zn ions (López-Millán et al., 2009).

Cadmium is a non essential element widely introduced to the soil by anthropogenic smelting and mining activities. The exposure of plants to high concentrations of Cd affects growth and development of plants. High levels of

Cd causes visible injuries like chlorosis, growth inhibition, browning of root tips and finally leads to death of the plants (Wojcik and Tukiendorf, 2004). Cadmium induces the generation of reactive oxygen species (ROS) and thus cause lipid peroxidation, indicated by higher accumulation of malondialdehyde (Singh and Shah, 2014). Cadmium negatively impacts the light use efficiency and it significantly damages the photosystem I and II and down regulates their activities (Faseela et al., 2019; Song et al., 2019). On the other hand, being an essential element Zn turns to be toxic to plants only when its concentration exceeds the tolerance limit. Over accumulation of Zn in plants, induces detrimental effects; it causes metabolic imbalances, affects the thylakoid electron transport and decreases membrane permeability (Rout et al., 2019). Zinc also triggers the overproduction of ROS causing oxidative stress due to the imbalance in the antioxidation machinery (Cui and Zhao, 2011). Elevated Zn concentrations also affect the photosystem II (PSII) efficiency and electron transport in noncyclic photophosphorylation (Szopiński et al., 2019). As per the previous reports, Cd and Zn can damage the water-splitting complex and thus reduce net electron flux to linear electron transport (Szopiński et al., 2019). Cadmium and Zn can also induce structural and functional modifications of the photosynthetic units, which affect its activity (Paunov et al., 2018). Replacement of divalent metals ions such as magnesium (Mg^{2+}) and manganese (Mn^{2+}) involved in photosynthesis by Cd and Zn affects the chlorophyll biosynthesis and water oxidizing system of PSII (Küpper et al., 2002). Cd and Zn are reported to cause a reduction in the activity of rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase), which reflects in the net productivity of the plant.

Oryza sativa and *Zea mays* are widely grown cereal crops and more than half of the world population depends on these crops for daily food consumption. In the present scenario, efforts are being taken to enhance the global production of these crops to cope with the demand of the increasing

population (Kong et al., 2018). According to the report of United States Department of Agriculture (USDA), the world production of *O. sativa* for the year, 2020/2021 will be 501.20 million metric tons (USDA, 2020). Currently, nearly 1147.7 million metric tons of *Z. mays* is being produced together by over 170 countries from an area of 193.7 million ha with an average productivity of 5.75 t/ha (IIMR, 2020). At the same time, many reports have indicated an alarming concentration of heavy metals in the arable lands and the farmers are forced to use these contaminated lands to cultivate these crops (Kong et al., 2018; Mishra and Mishra, 2018). The productivity of *O. sativa* and *Z. mays* plants are adversely affected by exceeding concentrations of heavy metals (Fahad et al., 2019). Thus, the higher Cd and Zn concentration in the agricultural fields is a concern for *O. sativa* and *Z. mays* cultivation (Shahid et al., 2015; Fahad et al., 2019). Further, these metals cause imbalance in the ionic homeostasis of cells resulting in the accumulation of ROS, which induces oxidative stress in plants and ultimately ends up in reduced plant growth and productivity (Islam et al., 2014; Anjum et al., 2015).

Introduction of novel strategies to mitigate the Cd and Zn stress intensity in *O. sativa* and *Z. mays* growth can be considered as the need of this hour. *Oryza sativa* and *Z. mays* are tolerant to heavy metal stress and their tolerance potential were detailed in different earlier studies (Wuana and Okieimen, 2010; Vigliotta et al., 2016; Shao et al., 2017). *Zea mays* plants has the ability to extract Cd, Zn, Pb, and Cu from the contaminated land, similarly *O. sativa* has the potential to accumulate metals such as Cd, Zn, As, Pb, Cu and Hg (Wuana et al., 2010; Shao et al., 2017). However, the heavy metals accumulated in the roots of *O. sativa* and *Z. mays* decreased the productivity of these crop plants (Cai et al., 2020; Devi and Kumar, 2020). Moreover, Cd and Zn toxicity significantly reduces the seed germination of these plants and thus establishment of these plants in the contaminated agricultural land

becomes difficult (Bashmakov et al., 2005; Siddiqui et al., 2019). So, it is essential to find a strategy to enhance the cultivation and productivity of *Z. mays* and *O. sativa* with reduced metal uptake, while these are being grown in a contaminated land.

Once introduced into the environment HM will persist there, so one of the finest strategies to control HM stress in plants is to reduce its bioavailability and mobility towards plants. Even though detoxification and bioleaching are found to be effective in clearing the heavy metal contaminated soil, the increased chemical application, cost and labour make it an economically non feasible method. Therefore, it is essential to come up with a high efficient, cost effective and farmer friendly method (Mani and Kumar 2014). Bioremediation is an efficient method and it is the utilization of living organisms in the transformation of xenobiotics into less toxic or non toxic forms (Vidali, 2001). In bioremediation, metabolic activities of different microbes are supporting in the biotransformation of different inorganic contaminants. Among different bioremediation methods, mycoremediation with arbuscular mycorrhizae is considered as the fastest and effective method due to its ability to increase root area and thus facilitating enhanced HM absorption (Chibuike, 2013).

Arbuscular mycorrhizal (AM) fungi are obligate biotrophs which establish a mutually beneficial association with the root system of many terrestrial plants (Turk et al., 2006). AM association highly benefits the plants, as it improves the growth and yield by enhancing the mineral and water uptake (Berruti et al., 2015). AM also protects plants from the attack of soil-borne pathogens and improves the hardening of micro propagated seedlings (Gonzalez-Chavez et al., 2004; Rilling and Mummy, 2006; Ferrol et al., 2016). Moreover, the mycorrhizal association help the plants to overcome

many abiotic stresses like drought, salinity, flood, heat, heavy metal toxicity *etc*.

The AM association improves the metal tolerance potential of the plants to which they are associated with. The extensive hyphal network of AM fungi blocks the metal uptake from soil and its translocation from the root to shoot (Kaldorf et al., 1999). The external mycelium is able to produce glomalin, a glycoprotein which is having heavy metal binding properties (Agely et al., 2005). AM fungi interact with host plants and increases the antioxidant level which aid in ROS scavenging (He et al., 2007). Similarly, AM fungi decrease the effect of HM toxicity by producing phytochelatins and glutathione, which aid in the complexation of the toxic metal ions (Garg and Aggarwal, 2011). Fungal vesicle helps in further detoxification mechanism by sorting toxic compounds (Gohre and Paszkowski, 2006). Colonization of AM fungi, *Glomus mosseae* alleviated metal toxicity in *Cajanus cajan* (L.) Millsp. by decreasing metal absorption from soil, controlled oxidative stress and improved antioxidant defense mechanism (Garg and Aggarwal, 2012).

AM fungus are able to maintain an efficient symbiosis with *Canavalia ensiformis* plant growing in soil with high Cu concentration (Andrade et al., 2010). It was proved that *Oryza sativa* associated with AM fungus showed increased Cu binding capacity in root cell wall and thus reduced Cu uptake to the shoot system (He et al., 2009). In *Z. mays*, HM are selectively retained in the inner parenchyma cells associated with fungal structure (Kaldorf et al., 1999). AM association make *Plantago lanceolata* tolerant towards Cd toxicity by changing its polyamine metabolism (Paradi et al., 2003). These polyamines upregulates the activity of antioxidant enzymes that aid to control the oxidative stress elicited by the metal toxicity. *Z. mays* plant decreases accumulation of Pb in shoot and root at lower concentrations by AM symbiosis. The techniques like energy dispersive x-ray analysis (EDXA),

laser microprobe mass analysis (LAMMA), and secondary ion mass spectrometry showed that heavy metal tolerance in *Z. mays* is due to selective immobilization of HM in root tissues containing the fungal cells (Kaldorf et al., 1999).

Mycorrhization improves the metal tolerance potential of the host plant by eliciting morphological, biochemical and physiological changes in the plant. Induction of root hair formation by the elicitation of auxin biosynthesis, enhancement in the shoot biomass production, augmentation of water and mineral uptake, elevated antioxidant defense and accumulation of metabolites are the major mycorrhizal induced metal tolerance methods adapted by the plants (Janeeshma and Puthur, 2020). All these modification aid to improve the metal tolerance potential, but simultaneously the transfer of these xenobiotics to the edible portions of *O. sativa* and *Z. mays* have to reduced. Mycorrhization improves the phytostabilization potential of these crop plants by immobilizing metal ions in rhizosphere or root tissues. In the case of *Z. mays*, mycorrhization reduced the Cu uptake from the soil and prevented the translocation from root to shoot. This was achieved by the uptake and stabilization of the metal in the root tissues (Wang et al., 2007).

This study focus on the mycorrhizal mediated heavy metal tolerance mechanisms and the influence of this symbiotic association on the physiological and metabolic status of host plants exposed to Cd and Zn stressors. Moreover, this work evaluates the mycorrhizal mediated Cd and Zn immobilization in *O. sativa* and *Z. mays* plants that protect the shoot system. The findings of this study will help to understand the mycorrhizae associated phytostabilization of Cd and Zn in *Z. mays* and *O. sativa* by avoiding the accumulation of these toxic ions in the shoot.

Objectives of the present study includes,

1. To examine the differential growth kinetics of AM fungus in the roots of *Z. mays* and *O. sativa* subjected to Cd and Zn stresses.
2. Assessment of growth responses in non-AM and AM plants of *Z. mays* and *O. sativa* on exposure to Cd and Zn stresses
3. Evaluation of the modulation in the photochemistry of *Z. mays* and *O. sativa* associated with mycorrhiza and exposed to Cd and Zn stresses.
4. To validate the metabolomics in the roots and leaves of *Z. mays* and *O. sativa* in asssociation with mycorrhizae on exposure to Cd and Zn stresses.
5. Understanding the role of mycorrhizae in alleviating the intensity of oxidative stress induced by Cd and Zn stresses in *Z. mays* and *O. sativa.*
6. Evaluation of the structural remodulation and differential distribution of Cd and Zn in the leaves and roots of *Z. mays* and *O. sativa* associated with mycorrhizae.

REVIEW OF LITERATURE

2.1 Heavy metal toxicity

Heavy metal toxicity is caused by acerbic elevation of high density metals and metalloids like mercury (Hg), cadmium (Cd), arsenic (As), cobalt (Co), chromium (Cr), thallium (Tl), copper (Cu), nickel (Ni), zinc (Zn) and lead (Pb) in the environment which have adverse effects on plants, animals and many microorganisms (Guo et al., 2017). When the concentration of heavy metals exceeds beyond the relative environmental quality standards, it become hazardous to all living organisms and their existence. Thus, heavy metal toxicity is a serious concern of the century as it negatively impact on human health and growth of other organisms. Rapid industrialization and urbanization has increased the chances of heavy metal exposure. Major industries significantly contributing towards HM contamination includes textile, dye, electroplating, spare parts, metal coating, chemical fertilizers (Dhaliwal et al., 2020). Another source of HM contamination is the old mines and smelters from which the toxic xenobiotics like Cd is mined along with different unrefined ores; Cd is then removed and released into the environment during further purification process of the ores and this discharged HMs will runoff to different area which progressively contaminate the soil (Arao et al., 2010). Moreover, continuous application of chemical fertilizers especially superphosphate and superphosphate-based compounds with trace amount of HMs increases the risk of contamination in agricultural land. The accumulations of essential metals like Zn are exceeding the maximum permissible levels due to the excessive use of fungicides and pesticides by farmers. Irrigation of soil with metal contaminated wastewater and continues application of organic fertilizers like sewage-sludge fertilizer increases the chances of heavy metal exposure in agricultural lands (Arao et

al., 2010). Usually underground water is contaminated with HMs, utilization of this contaminated water for different purposes progressively increases the bioavailability and mobility of toxic metals (Nyambura et al., 2020).

The intensity and the mechanism of toxicity are different for each metal ion. Based on the increasing toxicity, the heavy metals can be orderly arranged as $Ag^{+} > Cd^{2+} > Hg^{2+} > T1^{+} > Cu^{2+} > Ni^{2+} > Zn^{2+} > Co^{6+} > Cr^{6+} > As^{3+} > As^{5+}$ (Naumann et al., 2007). The level of toxicity imparted by each metal was determined based on the concentration required for inducing 50% growth inhibition in *Lemna minor*. The selected parameters for this study were fresh weight, dry weight, chlorophyll *a*, chlorophyll *b* and total carotenoid contents, due to the high vulnerability of these variables towards HM toxicity (Naumann et al., 2007). Copper, iron, manganese, molybdenum, nickel, and zinc are essential micronutrients for plant growth; in higher concentrations these metals causes toxicity to plants and negatively impacts plant growth. Cadmium, lead, chromium, mercury, cobalt, gold, silver *etc.* are non essential elements to plant and are toxic even at lower concentrations. Different heavy metals have different effects on plants and soil. The major effects of heavy metal toxicity are toxic free radical accumulation that leads to lipid peroxidation and membrane leakage (Małkowski et al., 2019).

Different HMs negatively affects the normal metabolism of plants in different ways. The non-essential HMs such as Pb, Hg, Cr, As and Cd are rapidly concentrating in different levels of food chain and will become hazardous to living organisms. High rate of uptake and accumulation of these toxic metal leads to visible injuries and physiological disorders in plants (Zhou et al., 2008; Mahbub et al., 2017; Anawar et al., 2018; Srivastava et al., 2021). They bind to the water channel protein, which induces stomatal closure and it impairs the uptake of water by the root tissues (Zhang and Tyerman, 1999). These metals could damage enzymes, polynucleotides, transporters,

and cell membrane (Patra and Sharma, 2000). Cell cycle impairment and DNA damage were also observed during HMs toxicity (Azevedo et al., 2018). Moreover, metal ions induce the accumulation of H_2O_2 and MDA content in a cell that results in the development of oxidative stress (Chen et al., 2017). Reduction in photosynthetic pigments, tissue moisture content, and growth rate are also associated with HMs toxicity (Sharma and Dubey, 2005; Chen et al., 2017; Rafiq et al., 2018).

The excess accumulation of different essential elements like Cu, Ni, Co, Zn and Fe become toxic to plants, which leads to plant growth retardation and leaf chlorosis (Lewis et al., 2001; Aly and Mohamed, 2012; Lwalaba et al., 2017; Khan et al., 2020; Tadaiesky et al., 2021). The excess metal ions can reduce the rate of photosynthesis, pigment synthesis, and membrane integrity and prevent efficient photosynthetic electron transport, resulting in the accumulation of different free radicals, which induce oxidative stress in plants (Fernandes and Henriques, 1991; Li et al., 2009; Zaid et al., 2019; Saaltink et al., 2017; Chen et al., 2021). The increased levels of electrolyte leakage and overaccumulation of H_2O_2 content is considered as an indicator of the oxidative stress induced by different HMs (Valivand and Amooaghaie, 2021). As a result of this oxidative stress, extreme lipid peroxidation and associated membrane degradation was observed in plants exposed to HMs stress (Janas et al., 2010). Moreover, higher concentration of Cu can modify the structural integrity of thylakoid (Panda, 2008).

Among these metals, Cd and Zn are potentially toxic to plants as well as animals due to their increased bioavailability (Janeeshma et al., 2021b).

2.1.1 Cadmium toxicity

Cadmium (Cd) is one of the most hazardous environmental pollutants. As a result of human activities such as mining, smelting, application of

insecticides, pesticides and conventional waste disposal technologies, Cd accumulation drastically exceeds its regulatory limit in agricultural soil (100 mg/kg soil). The high toxicity and large solubility of Cd makes it a potential pollutant in water (Pinto et al., 2004). Cd toxicity induce reduction in photosynthesis, water uptake and nutrient uptake by plants.

High levels of Cd causes visible injuries like chlorosis, growth inhibition, browning of root tips and finally death of the plant (Wojcik and Tukiendorf, 2004). As in the case of other heavy metals, Cd induces the generation of reactive oxygen species (ROS) and cause lipid peroxidation, indicated by higher accumulation of malondialdehyde (Tran and Popova, 2013). Cadmium affects photosynthesis by the down regulation of photosystem II (Huang et al., 2017).

Cadmium also affects the mineral composition and uptake of different plants. Cadmium reduces the uptake of Mn and Cu and it also reduces the biosynthesis of chlorophyll molecules in the leaves of *Brassica juncea.* (Salt et al., 1995). Study on *Z. mays* root indicated that the Cd toxicity induces more unsaturated fatty acid compositions and increased level of phosphatidyl choline, phosphatidyl ethanol amine *etc*. Cadmium induces the production of H_2O_2 which plays important role in the cell wall stiffening process and it would inhibit the root growth. It was observed that Cd toxicity reduces root growth in maize seedlings (Jamal-Abad and Rahmatzadeh, 2007). It was reported that external application or priming of maize seedling with salicylic acid reduces the oxidation effect caused by Cd toxicity (Al-Mureish et al., 2014).

2.1.2 Zinc toxicity

Zinc (Zn) is an essential element, but due to excessive use of agro-chemicals, excess Zn is contaminating the soil. Sewage sludge deposition,

fertilizers, emission from municipal wastes *etc*. are also the primary cause for the introduction of Zn into the soil. Higher concentration of Zn results in growth retardation and chlorosis (Janeeshma et al., 2021a). Another negative effect of Zn toxicity is the appearance of purplish red color in leaves, which indicates phosphorous deficiency (Nagajyoti et al., 2010). Zinc reduces the activity of rubisco by replacing magnesium and it directly reduces the rate of photosynthesis (Rout and Das, 2009). Moreover, this metal inhibits thylakoid electron transport, resulting in the reduction of photosynthesis (Janeeshma et al., 2021a).

At toxic level Zn will bind to the plasma membrane, leading to reduced water uptake (Rygol et al., 1992). Moreover, Zn induced an oxidative stress in plants by the accumulation of ROS and that acts as a reason for MDA accumulation and membrane degradation (Janeeshma et al., 2021a). Different plants have different level of tolerance towards Zn toxicity. According to the study conducted by Arora et al. (2008), *Z. mays* is a plant with high level of tolerance to zinc toxicity in soil.

2.2 Heavy metal toxicity in agricultural land

The impact of heavy metal toxicity on mankind increases along with an increase in the contamination of the agricultural lands with different toxic metals and metalloids (Gashi et al., 2020). The accumulation of the xenobiotics in the organisms and biomagnification of these compounds increases the impact of toxicity on mankind. The term 'biomagnification' refers to the increase in heavy metal concentration towards the higher trophic level of food chain as compared to the lower trophic levels (Gray, 2002). Different developed, undeveloped and developing countries have reported extensive HMs contamination in the agricultural lands owing to the development of different sources of xenobiotics. Zanjan Province, one of the important area of food production in northwestern Iran was reported to have

heavy metal contamination (Askari et al., 2020). Askari and coauthors analysed the heavy metal content in this agricultural land and found that different heavy metals were exceeding the non toxic limit in the order Zn > Pb > Ni > Cu > Co > Cr > Cd, which amplifies the human health consequences, owing to the consumption of food crops that have been grown in these contaminated lands.

Agricultural lands in China was affected by heavy metal toxicity and according to the recent reports of national survey of soil pollution in China (NSPCIR), more than 23 million hectares of agricultural land were contaminated with different heavy metals (Xiang et al., 2020). Successive sludge applications in agricultural lands lead to the accumulation of heavy metals in United States and some west European countries (McGrath et al., 1994). Irrigation of agricultural lands with HM contaminated water is another important threat, which results in the accumulation of HM in edible portions of the crops. HM contaminated Akaki river water of Ethiopia was used to irrigate agricultural land. Most of the vegetables harvested from the land showed accumulation of Cd, Cr, Cu, Zn, Mn, Fe and Ni (Prabu, 2009). The same study reported that leafy vegetables showed prominent accumulation of Cd whereas the rate of Cd accumulation was low in non leafy vegetables (Prabu, 2009). Similarly, when the urban agricultural lands of Faisalabad, Pakistan was irrigated with the city effluent water, the vegetables grown in the area showed Pb, Zn, Fe and Cu accumulation (Ghafoor et al., 2004). Due to the excessive metal mining activities, the agricultural lands of Japan were also severely contaminated with different inorganic xenobiotics, especially Cd (Arao et al., 2010).

Atmospheric deposition and application of livestock manures and sewage sludge were reported as the major sources of HM contamination in the agricultural lands of England and Wales (Nicholson et al., 2003). Further,

contribution of livestock manures in the heavy metal contamination was analysed by Leclerc and Laurent (2017). They found that Hg, Zn and Cu are the major xenobiotics released from livestock manures to the agricultural soil and absorbed by crop plants. 137,000 km^2 of agricultural lands of European Union's was also contaminated with heavy metals (Tóth et al., 2016). In agricultural land, soil microbiota plays a significant role in the productivity of the crop plants. But, HM toxicity critically reduces the microbial population of the soil, which further reflects on the reduction in crop biomass (Giller et al., 1999).

2.3 Impact of heavy metal toxicity on staple foods production

Heavy metal free staple food production is the necessity of the current era and it is essential for the good quality crop production and health of the consumers. Therefore, it is essential to find solutions to reduce HM accumulation in the edible portion of the plants cultivated in contaminated agricultural lands. Many researchers have investigated the metal tolerance and phytostabilization potential of different hybrids and selected the candidates that can be cultivated in a metal contaminated land. Eshu10, cultivar of *Solanum tuberosum* that potentially reduced the HM translocation to the tubers, hence was selected to grow in the Cd contaminated agricultural land (Ye et al., 2020). An investigation conducted in the durum wheat found that a single dominant gene controls the trait 'low-Cd concentration'. But, this gene did not significantly alter the economic traits of the plant. Thus, incorporation of this allele to other crops such as sunflower, rice, and soybean was found to be a good strategy to decrease the heavy metal uptake (Grant et al., 2008). *Sorghum* is an important crop, worldwidely used for food, animal fodder and biofuels. It was reported that *Sorghum* have phytostabilization capacity to accumulate Cd and Zn primarily in root during HM stress (Soudek et al., 2014). Another food crop *Brassica campestrie* immobilized and accumulated

Cu, Zn and Ni and other HMs in the shoot instead of the root system (Barman et al., 2000), which significantly increases the chances of HM exposure to human.

The important staple food crops such as rice, maize, and wheat showed potential for metal accumulation and this could increase the risk of exposure of the metal to different trophic levels of the food chain (Grant et al., 2008). Wheat (*Triticum aestivum*) is a widely cultivated cereal for its seed, which is a source of multiple nutrients and dietary fiber. It showed pytostabilization of Fe, Ni, Cr, Pb and Cd by accumulating these HMs in root (Barman et al., 2000). Wheat also showed higher activities of various antioxidant defense enzymes and also proline accumulation, which are the important factors for phytoremediation (Ali et al., 2019).

Among the different staple foods, rice and maize are two important crops feeding almost half of the world population with high level of metal tolerance. Excess concentration of different heavy metals such as Cd, Pb, Ni and Cu significantly reduced the productivity of rice by hindering photosynthesis, cellular organelles functioning, and acquisition of mineral ions (Fahad et al., 2019). When rice plants were exposed to Cd toxicity, it elicited oxidative stress in the shoot system, which was reflected in the enhanced MDA, H_2O_2 and $O_2^{\cdot -}$ content. Moreover, Cd toxicity induced the expression of different transporters in rice plants including heavy metal ATPase (*OsHMA2* and *OsHMA3),* and natural resistance-associated macrophage proteins *(OsNramp1* and *OsNramp5)* (Chen et al., 2019). Similar results were found in the study conducted by Jiang and coworkers (2020) in rice plants exposed to Cd. Severe structural integrity loss of the organelles of rice plants exposed to Cd was observed with the enhancement in the gene expression of *OsLCT1* (low-affinity cation transporter) and *OsNramp5 (*Cui et al., 2017). The increase in the expression of these genes increases the level of

Cd uptake thus the plant encounters severe stress. Cd accumulation in the leaves of rice plants significantly reduces the rate of transpiration, stomatal conductance and carbohydrate production (Gao et al., 2018). The study also pointed out that the Cd content in the stem is the major factor determining the concentration of this metal in brown rice (Gao et al., 2018). Moreover, Cd toxicity caused a dramatic reduction in the grain yield and rice biomass (Hussain et al., 2020).

When rice plants were exposed to Zn toxicity, important physiological parameters were impaired. 200 μM of $ZnSO_4$ treatment resulted in the reduction of seedling biomass in rice plants (Gu et al., 2012). When two different rice cultivars of rice were treated with high concentration of Zn, it was noted that there was a significant reduction in root length, total root surface area and total root tip amount (Song et al., 2011). Based on the generation of different ROS species and rate of MDA accumulation cv. TY-167 was selected as Zn-resistant and cv. FYY-326 as Zn-sensitive varieties (Song et al., 2011). Moreover, high concentration of Zn induced an increase in the cell death and chlorosis in rice plants (Chang et al., 2005; Chen et al., 2017).

Similar to rice, maize also exhibited dramatic accumulation of different ROS species and extensive lipid peroxidation under heavy metal stress (AbdElgawad et al., 2020). Yathavakulasingam et al. (2016) reported an acceleration of lead phytostabilization capacity of maize grown in association with *Gliricidia sepia*. Maize immobilize heavy metals through adsorbtion on roots and precipitation within rhizosphere. A huge decline in the growth and yields of maize plants was observed under different metal treatments such as Cd, Cr and Ni (Rizvi and Khan, 2019). Of the three different treated metals Cd (36 mg/kg) showed most lethal effects and caused a reduction of root and shoot length. Additionally, maize plants showed reduction of total chlorophyll

content, grain yield and grain protein at the same toxicity level of Cd (Rizvi and Khan, 2019). Heavy metal induced reduction in the internodal length and leaf area of maize was also reported in a study conducted in the Department of Agronomy, School of Agriculture, Lovely Professional University, Phagwara (Devi and Kumar, 2020).

Under Zn toxicity, maize plants showed prominent reduction in the length of primary seminal root, leaf area, fresh and dry weight of root and shoot (Bokor et al., 2014). Similar to this, high Zn content induced oxidative stress in maize plants (Islam et al., 2014). Reduction in the biomass production of maize plants was also observed under Zn stress. Membrane permeability of maize plants was affected due to high concentration of Zn (Kaya et al., 2009).

2.4 Heavy metal tolerance mechanisms of plants

Usually plant shows different mechanisms to tolerate various stresses. Plants have mechanism to avoid, resist, escape, and endure environmental stresses. Crassulacean acid metabolism (CAM), leaf area reduction, accumulation of compatible solutes (proline, glycine betains *etc.*), osmotic adjustment, resurrection and heteroblasty are different stress tolerant mechanisms exhibited by plants. Most of the stresses induce the production of abscisic acid (ABA) content in plants.

Stress induce the accumulation of ROS in plants and the cellular damage caused by free radicals is generally prevented by antioxidant enzymes like superoxide dismutase (SOD), peroxidase (APX), catalase (CAT) and glutathione reductase (GR). To prevent the accumulation of ROS, SOD efficiently transform $O_2^{\cdot -}$ to less toxic H_2O_2, further APX convert H_2O_2 to water, using ascorbate as electron donor (Per et al., 2016). CAT and APX

plays an important role in preventing oxidative stress by reducing the level of H_2O_2 (Wang et al., 2015).

Cadmium resistance in plants is associated with the presence of SH-containing peptides phytochelatins (PCS) and glutathione GSH (Grill et al., 2007). Inhibition of PC synthesis leads to the reduction in Cd detoxification of the plants and it indicates significant role of this molecule in metal complexation and inactivation. After complexation of Cd with PCS, the complex form is sequestrated into the vacuole located (Vögeli-Lange and Wagner, 1996). Moreover, Cd is also accumulated in leaf trichomes as in the case of *Brassica juncea* (L.) Czern. (Salt et al., 1995).

Usually in Zn tolerant plants, Zn will bind to the cell wall, and thus it becomes less interfering with plant metabolism. Zn resistant ecotype *Dechampsia caespitosa* accumulate less Zn in apical parts of the roots, but more in the basal parts (Godbold et al., 1983). Inside the cell, Zn accumulates in vacuole. It was found *Thlaspi caerulescens* could tolerate 40 mg Zn g^{-1} (dry matter) by enhanced Zn influx into the roots and increased transport to the shoots, and this plant can be used in phytoremediation (Lasat et al., 1996).

Heavy metal induced prominent anatomical changes were observed in different plants. Cell wall thickening is one of the major anatomical responses found towards Cd and Zn stresses, especially the cell walls of epidermis, endodermis, xylem sclerenchyma and vessels exhibited cell wall thickening (Janeeshma et al., 2020). Histological modifications have been reported in different plants, such as maize (Vaculík et al., 2012a), rice (Huang et al., 2019), *Sorghum bicolor* (Kasim, 2006), *Arundo donax* (Guo and Miao, 2010), and *Pistia stratiotes* (e Silva et al., 2013) due to Cd and Zn toxicity. A significant reduction in vessel width and density was observed in *Cajanus cajan* exposed to Cd stress. Cell wall thickening, cell degradation, and crystal

formation have also been observed as responses of different plant candidates to metal stress (Sruthi and Puthur, 2019).

Additionally, theses heavy metals induced different molecular changes in plant tissue, which could be evaluated with the help of FTIR analysis. Through this technique metal induced modifications in the characteristics of the functional groups can be analysed (Sruthi and Puthur, 2019). Moreover, the interaction between the functional groups of different biomolecules and metal ions can be analyzed by FTIR. Therefore, FTIR data can be used as an indicator of the heavy metal stress in plant tissues via the functional group modification due to metal toxicity (Sruthi and Puthur, 2019).

Association of plants with different microorganisms boosts up the metal tolerance level of plant candidates (Tiwari and Lata, 2018). Plant-microbe interaction is a strategy to tolerate the toxic level of metal content in the soil. Even under extreme conditions like heavy metal contamination, these microbes promote the growth of different plant partners. Microbial mediated retrieval and reduction of heavy metals from water and polluted lands is known as bioremediation, which is a sustainable approach for clearing contaminated land (Verma and Kuila, 2019). Due to the high efficiency and cost-effectiveness, bioremediation is the paramount method for clearing heavy metal contaminated soil over other methods like detoxification and bioleaching (Mani and Kumar, 2014). In different bioremediation methods, mycoremediation with arbuscular mycorrhizae is considered as the fastest and effective method due to its ability to increase root area and this facilitates enhanced HM absorption (Chibuike, 2013). AM fungi mediated alleviation mechanisms of HM stress in plants is detailed below.

2.5 Arbuscular mycorrhizae (AM)

Arbuscular mycorrhizae association represents the mutualistic part of the symbiotic spectrum, where a bidirectional exchange of benefits occurs

(Shi et al., 2019). They are characterized by the formation of unique structures such as arbuscules and vesicles. A branched tree like structure called arbuscule develops into the cell by invagination of the plasma membrane of host, which are formed by repeated dichotomous branching of the hyphae. The function of the arbuscule is to increase the surface for metabolite exchange and so to enhance active transport of carbohydrate between the plasma membrane of host and the hyphae of the fungus (Smith et al., 1994). There should be a balance between the formation and the degeneration of arbuscules. The life span of a arbuscule is considered as 2.5-3 days (Alexander et al., 1988). The vesicle formation is another important feature of AM fungi. It acts as a storage organ in fugal partner and it can be formed within or between the cells.

Based on the phylogenetic analysis conducted by Spatafora et al. (2016), arbuscular mycorrhiza belongs to the phylum Mucoromycota and subphylum Glomeromycotina. The importance of arbuscular mycorrhizae depends on its host range because more than 85% of land plants can associate with this group of organism. This symbiotic association is a fascinating experience of co-evolution which appeared 400-460 million years ago when the first land plants emerged. The metal remediation properties of AM fungi were reported by many authors and therefore this organism in association with plants is being exploited to clear HM contaminated agricultural lands (Lokhandwala et al., 2017; Rozpądek et al., 2014; Zhang et al., 2019).

2.6 HM remediation mechanisms by plants associated with AM

Phytoremediation can be achieved by different mrthods like phytofiltration, phytovolatilization, phytodegradation, rhizodegradation, phytodesalination, phytoextraction and phytostabilization. Phytofiltration is the removal of pollutant from contaminated water surface to avoid its movement to underground water, with the help of plant root or seedlings or

excised shoot (Mukhopadhyay and Maiti, 2010). Phytovolatilization is used to convert organic pollutant and heavy metals in the soil to its volatile forms using plants. Ulhassan et al. (2019) reported that *Brassica* sp. had the capacity to uptake and transpire selenium (Se). Phytodegradation is the degradation of organic xenobiotics like synthetic herbicides and insecticides with the help of enzymes released by plants such as dehalogenase and oxygenase. Rhizodegradation is a process of breakdown of organic pollutants in the soil with the help of microorganisms in the rhizosphere. *Pseudomonas, Arthrobactor, Agrobacterium, Alcaligenes, Azetobactor, Mycobacterium, Bacillus, Clostridium* and *Micrococcus* are the major bacteria involved in rhizodegradation (Andrade et al., 1997). Major fungi observed in rhizosphere includes *Fusarium, Verticillium, Aspergillus* and *Penicillium.* Whereas phytodesalination is a technique where halophytic plants are used for removal of sodium ions from the soil (Hasanuzzaman et al., 2014).

However, phytoextraction and phytostabilization are the major phytoremediation methods that have been reported in plants with AM association and it was recapitulated in Table 1 (Janeeshma and Puthur, 2020). Phytostabilization is the immobilization of heavy metals in the root system of plants by absorption, precipitation and reduction without its accumulation in the shoot system (Radziemska et al., 2017; Shackira and Puthur, 2017). It prevents the leaching of heavy metals and thus groundwater contamination is also reduced. Phytostabilization turns to be a fine and more beneficial method when we consider the cultivation of crops in heavy metal contaminated soil and HM can be restricted to the root region without being translocated into the shoot and yield producing parts of the plants. Phytostabilization properties is analysed using bioconcentration factor (BCF) and translocation factor (TF) and it was calculated by the equations,

Table 1: Host-mycorrhiza mediated phytoremediation mechanisms operating under various metals.

Mycorrhizae	Host plant	Heavy metals	Mechanisms
Glomus intraradices	*Helianthus annuus*	Cr	Phytoextraction
Glomus mosseae and *G. caledonium*	*Holcus lanatus*	Ar	Phytostabilization
Acaulospora sp. *Glomus* and *Scutellospora* sp.	*Plantago lanceolata*	Cd	Phytostabilization
Glomus mossea, G. caledonium, G. claroideum and *G. intraradices*	*Thymus polytrichus*	Zn	Phytoextraction
Glomus deserticola and *G. mosseae*	*Eucalyptus globulus*	Pb	Phytoextraction
Suillus bovinus	*Pinus sylvestris*	Zn	Phytoextraction
Glomus mosseae	*Coreopsis drummondii, Pteris vittata, Lolium perenne, Trifolium repens*	Cu	Phytostabilization
Glomus intraradices, G. mosseae, G. claroideum, and *G. geosporum*	*Nicotiana tabacum*	Cd	Phytostabilization
Acaulospora laevis Gigaspora margarita,	*Astragalus sinicus*	Lanthanum	Phytostabilization

Glomus intraradices			
Glomus intraradices	*Zea mays*	Pb	Phytostabilization
Acaulospora mellea	*Zea mays*	Cu	Phytoextraction
Glomus intraradices	*Agrostis capillaris*	Pb, Zn, Cu, Cd	No effect
Glomus mosseae	*Trifolium repens*	Cd, Mo, Mn, Zn,Cu, Al, As,Ni	Phytostabilization
Suillus luteus	*Pinus sylvestris*	Cd	Phytostabilization
Glomus deserticola and G. claroideum	*Eucalyptus globulus*	Ar	Phytoextraction
Glomus deserticola	*Eucalyptus globulus*	Zn	Phytoextraction
Glomus mosseae G. versiforme	*Zea mays Sorghum bicolor*	rare earth elements La, Ce, Pr, Nd	Phytostabilization
Glomus mosseae	*Cajanus cajan*	Cd, Pb	Phytostabilization
Glomus mosseae	*Glycine max*	Mn	Phytostabilization
Glomus intraradices G. spurcum	*Sorghum bicolor*	Cu Zn	Phytostabilization Phytoextraction
Glomus mosseae or *G. intraradices*	*Populus alba, Populus nigra*	Zn	Phytostabilization
Glomus mosseae	*Glycine max, Lens culinaris*	Zn, Ni	Phytoextraction
Glomus intraradices	*Helianthus annuus*	Cd	Phytostabilization
Glomus mosseae G. intraradices	*Capsicum annum.*	Cr	Phytostabilization

$$\text{Bioconcentration factor (BCF)} = \frac{\text{Concentration of metal in root}}{\text{Concentration of metal in soil}}$$

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

A considerable reduction in the availability and mobility of copper in the soil was found to be refrained by phytostabilization (Radziemska et al., 2017). Selected plant species such as *Festuca rubra* and *Acanthus ilicifolius* were used for this purpose and they were characterized by a rapid increase in biomass production under HM stress (Simon, 2005; Shackira and Puthur, 2017). Generally, metal excluders exhibited low TF value (TF < 1) which indicates the reduced upward movement of HMs. One of the major metal excluder *Hibiscus cannabinus* immobilized 16.55 ± 4.11 mg Pb in its root, which had an average biomass of 10.18 ± 1.85 g per plant (Ho et al., 2008). Plants used for phytostabilization are characterized by metal tolerance, but necessarily need not be a hyperaccumulator (Mendez and Maier 2008). Along with all other common tolerance mechanisms, enhanced cell wall biosynthesis, inactivation of the metal at rhizosphere and its accumulation in the root are specific to phytostabilizers. Metal stabilization properties of plants are enhanced when they are associated with AM fungi (Hutchinson et al., 2004; Zhang et al., 2019). The importance of AM fungi in phytostabilization was deeply investigated in different grass species by Regvar et al. (2006). They observed that when gradual replacement of non-mycorrhizal plant with mycorrhizal plant in polluted lands was done, the metal stabilization potential of the plant was increased. Chen et al. (2003) observed that clover plants associated with mycorrhizae increased the retention of Zn in the root system and thus prevented its translocation into the shoot. All these studies point towards AM induced enhancement in the phytostabilization strategies of the host plant.

Phytoextraction is the most prominent method in phytoremediation which facilitates the uptake of HM from soil and its immediate transport to the shoot system. Radziemska et al. (2017) reported phytoextraction as the most efficient phytoremediation method to clean up the contaminated soil because HM can be removed together with plant upon harvest and can further be processed through phytomining for recapturing the elements. The high rate of biomass production (specifically shoot biomass) with an intensively branched root system and higher tolerance, accumulation and translocation potential of heavy metals to the shoots are the characteristics of the plants used in phytoextraction. Plants fulfilling all these properties are included in a particular class known as hyperaccumulators or metallophytes. Growing hyperaccumulators were considered as a natural process of phytoremediation and it can avoid leaching of HM to deep soil, but most of the natural hyperaccumulators have shown a low rate of biomass production and this drawback of these plants can be overcome by growing AM fungi in association with the respective plants. Generally, exudation of organic acids by AM favors phytoextraction by inducing the metal bioavailability from soil through mechanisms like acidification, chelation and redox reactions (Teotia et al., 2016). Arriagada et al. (2005) observed that AM associated *Eucalyptus*, a hyperaccumulator plant showed increased uptake of Cd in their shoots, and they also studiedon the ability of *Glomus deserticola* to remove a high quantity of Cd from the soil than compared to other *Glomus* sp. Interestingly, they reported that the redistribution of Cd and Pb was higher in stems as compared to that of leaves of *Eucalyptus*, possibly a means to avoid the interaction of HMs with many important metabolic pathways occurring in leaves. Leung et al. (2006) reported mycorrhizal stimulated enhancement of arsenic uptake in *Pteris vittata*, it was a strong evidence for the mycorrhiza induced enhancement in the phytoextraction potential of a known

hyperaccumulator. An overview of mycorrhizal mediated HM stress alleviation is represented in Fig. 1.

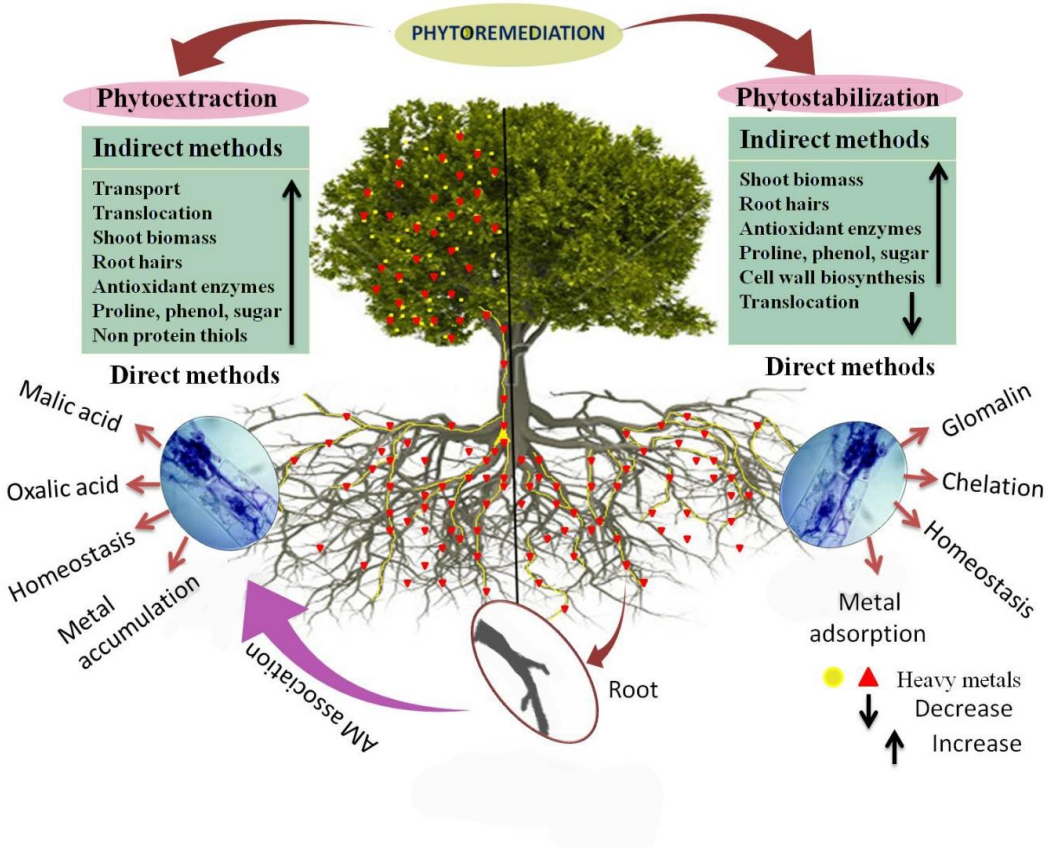

Figure 1: The role of mycorrhizal mediated direct and indirect mechanism in phytoextraction and phytostabilization of heavy metals.

2.6.1 Direct influence of AM on heavy metal tolerance

Mycorrhizae have their own mechanisms to overcome metal stress and they operates in a host independent manner. It was achieved by chelating HM inside the fungal structures and immobilizing the metal in mycorrhizosphere with the help of the protein glomalin (Schindler et al., 2007). The direct involvement of mycorrhizae in HM tolerance was represented in figure 2.

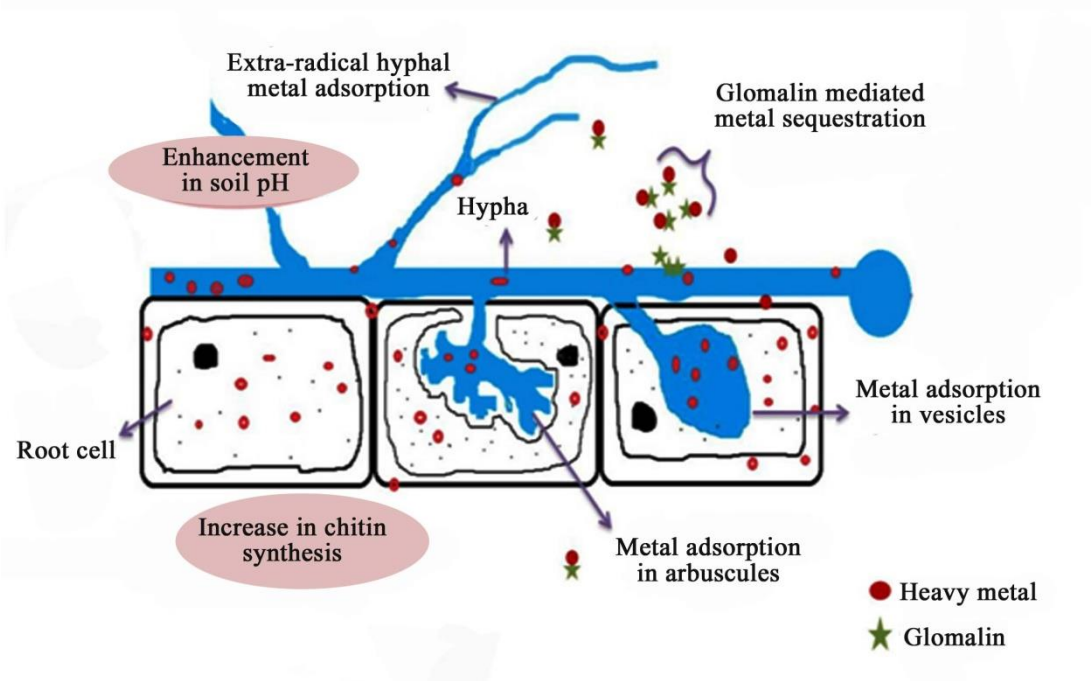

Figure 2: An overview of direct metal tolerance mechanisms in AM

2.6.1.1 Immobilization of metal in mycorrhizosphere with glomalin

AM associated plants could better handle the HM in the soil with the help of external mycelium by producing glomalin, a glycoprotein which is having metal binding properties. There is a strong correlation observed between the content of glomalin in the soil and the quantity of bounded HM. Gonzalez-Chavez et al. (2004) reported that glomalin was the first cellular component of AM coming in contact with ions in the rhizosphere. Mycorrhizal fungi released glomalin into the soil. Glomalin Related Soil Protein (GRSP), a complex form of glomalin has been identified using monoclonal antibody MAb32B11, raised against crushed spores of *Glomus intraradices* (Wright, 2000).

Glomalin is a persistent N- linked glycoprotein assumed to have an array of aminoacids such as asparagine, serine, threonine or hydroxylysine which are covalently bound to different carbohydrate residues (Schindler et al., 2007). Schindler and collegues (2007) tried to characterize glomalin using

spectrtal specificity, but Gadker and Rillig (2006) tried for the same by adopting molecular approaches. During the studies, Gadker and Rillig (2006) found a sequence similarity between glomalin and heat shock protein 60 (hsp 60) which helps to overcome the major limitations to the glomalin related research *i.e*, its unknown identity.

Glomalin had the potential to complex with HM and this complex could be later extracted from the contaminated soil. It was reported that 1g glomalin could extract up to 4.3 mg Cu, 0.008 mg Cd and 1.12 mg Pb from polluted soil (Gonzalez-Chavez et al., 2004). *Gigaspora rosea* sequestered very high levels of Cu with the help of glomalin, to the extent of 28 mg Cu per gram glomalin (Göhre and Paszkowski, 2006). The decomposition rate of glomalin and glomalin-metal complex was analysed in Cd and Pb contaminated soils and the results implicates to the higher relative stability of GRSP which reduces the bioavailability of these toxic metals to plants (Wu et al., 2014). This glomalin secreted by AM hyphae helps for the complexation of metal and convert it into inactive form and thus protect the plants from the direct exposure of toxic metal ions.

2.6.1.2 Accumulation of metal in fungal structures

Kaldorf et al. (1999) focused on the ability of AM fungal structures to accumulate different toxic metals. They reported stabilization of Zn and Ni in the inner cortical cells of *Zea mays* roots, where arbuscules and intraradical hyphae were located. They proved this hypothesis by combining different techniques like secondary ion mass spectrometry (SIMS), energy dispersive X-ray analysis (EDXA) and laser microprobe mass analyzer (LAMMA). Weiersbye et al. (1999) reported that absorption and localization of HMs and radionuclides in AM structures will limit the metal availability to the host plant. Metals like Mn, Cu, Ni and uranium (U) were accumulated in the vesicles of mycorrhizae. The fungal vesicles in mycorrhizal roots showed

increased U accumulation, which prevents the transport of this element to shoot. They also observed sequestration of HMs by mycorrhizal spores which were isolated from gold (Au) and U mine tailings. Experiments of Joner and Leyval (1997) conducted in compartmented pots proved that extraradical hyphae of *G. mossae* transported Cd from the soil, but prevent the movement of HM from fungus to plant by immobilizing the HM within the AM itself. The ability of AM to localize HM in fungal mycelium was also supported by Zhang et al. (2019). This direct involvement of mycorrhiza in the metal precipitation helps to reduce the availability of metal ions to plants and thus reduces the chance of biomagnification of these metals in humans through food chain.

2.6.1.3 Adsorption of HM by extraradical hyphae

According to Colpaert and Van-Assche (1993), Cd treated mycorrhizal *Pinus sylvestris* showed evidence of positive correlation of AM induced phytoremediation with extraradical mycelium produced by it. These extraradical hyphae were exhibiting very strong metal binding capacity (Christie et al., 2004). The unusually high HM adsorption capacity of extraradical hyphae attributed phytoremediation potential to plants associated with AM (Joner et al., 2000; Chen et al., 2003). Leyval et al. (2002) reported that this hyphal mediated metal accumulation helps to immobilize metal ions. Joner et al. (2000) reported the strong binding capacity of HMs by extraradical hyphae of different isolates of *Glomus* sp. which was incubated in a solution containing metal for 6 h. The adsorption of metal ions on hyphae appeared within 30 minutes and Zn adsorption by hyphae ranged from 5.6 to 76 mg g^{-1}. Similarly, crystallization of Zn in the AM hyphae was reported by Khan et al. (2000). Using a modified glass bead compartment cultivation system, Chen and coworkers (2003) observed that Zn concentration in extraradical hyphae of *Glomus mosseae* was up to 1200 mg kg^{-1}, nearly 10

times higher than that of the corresponding roots. Hyphae of *G. intraradices* associated with roots of *Daucus carota* had higher uranium flux rates and higher uptake than host roots (Rufyikiri et al., 2003). Hrishikesh et al. (2010) reported that the extensive mycorrhizal hyphae act as the sink of HM in contaminated soil and it was supported by the high adsorption efficiency reported for the same. These extraradical hyphae mediated metal sequestration reduces its bioavailability in rhizosphere and helps the plants to survive in high metal contaminated area.

2.6.1.4 Adsorption of HM to chitin

Phytostabilization properties of AM increased due to the presence of chitin and chitosan in its cell wall and binding of HM to these polysaccharides depends on the strength of the amino acids integrated with cell wall (Galli et al., 2003). Chitin and chitosan mediated uptake of Cr (III) and Cr (VI) was evaluated and chitosan was reported as a strong adsorbent of Cr (Maruca et al., 1982; Saravanan et al., 2013). The results of the study conducted by Babel and Kurniawan (2003) suggests to use chitosan to clear Hg, Cr and Cd contaminated water due to its high adsorption capacity. It was proved that chitin extracted from mycelia of a non mycorrhizal fungus *Cunninghamella elegans* had the ability to accumulate copper and lead (Franco et al., 2004). Mycorrhizal association also have a very important role in phytostabilization by enhancing the passive adsorption of HM to the chitin of hyphal cell wall (Joner et al., 2000). Passive adsorption of HM facilitated by AM cell wall reduces the concentration of metal ions in rhizosphere as well as in plant cells.

2.6.2 Indirect influence of AM on host mediated heavy metal tolerance

Modifications in the morphology and physiology of host plant under the influence of AM association helps the plants to survive under HM stress. Reduction in root biomass, increased shoot biomass due to plant growth,

enhancement of water and mineral uptake, elevated antioxidant defense and accumulation of metabolites are the major mycorrhizal induced metal tolerance methods adapted by the plant.

2.6.2.1 Influence on root morphology

The root system is the first organ of plants in contact with HMs and it helps the absorption and accumulation of HMs. The enhanced absorption capacity can be considered as the major strategy for phytoextraction. Most of the hyperaccumulators are characterized by less root biomass (Khan et al., 2000). Mycorrhizal association stimulates the host to increase root hair formation and this leads to enhancement in root surface area for the acquisition of nutrients and HMs (Jastrow and Miller, 1993; Mohammadi et al., 2011). Mycorrhizal induced root development helps the *Chrysopogon zizanioids* to tolerate arsenic stress in soil and water systems (Caporale et al., 2014).

Many reports have pointed out the ability of AM to change the root architecture of the host during infection (Berta et al., 1995). Kaldorf and Ludwig-Muller (2000) supported it by demonstrating the impact of AM in influencing root morphology. Thanuja et al. (2002) reported that *Acoulospora laevis, Gigaspora margarita* and *Glomus fasciculatum* increased rooting percentage in the orthotropic cutting of pepper cultivar panniyur-1. AM induced plasticity in root morphology is mainly achieved by fine root hair formation. These changes in root morphology or architecture are achieved through the influence of AM in manipulating the phytohormone levels in the plants. *Glomus intraradices* associated with *Z. mays* increased phytohormone Indole-3 butyric acid (IBA) which was responsible for the root hair formation (Kaldorf and Ludwig-Muller, 2000). The role of AM to enhance the conjugated IBA content within the plant was also reported by Fitze et al. (2005). AM induced root hair formation aid the plant to prevent the metal

translocation to shoot by localizing the accumulated metals more in the root, and this strategy increase the tolerance of host plant towards HM.

2.6.2.2 Enhanced shoot biomass

The efficiency of HM extraction depends on the rate of shoot biomass production by the plants exposed to metal stress (Göhre and Paszkowski, 2006). AM associated plants showed an increase in shoot biomass production as compared to non AM plants. According to Turnau and Mesjasz-Przybylowicz (2003), the increased biomass production in different members of Asteraceae leads to an enhanced accumulation of Ni in AM plants as compared to non AM plants. Liu et al. (2005) reported that AM association can impart phytoremediation potential to a non hyperaccumulator plant by increasing its biomass. The enhanced rate of biomass production in AM associated sunflower plant resulted in a greater rate of Cr extraction and its uptake (Davies et al., 2002). Enhanced shoot biomass of AM plants during metal toxicity indicates high metal tolerance level of these plants and it could be exploited in the field of phytoremediation.

2.6.2.3 Role of plant cell wall in HM sequestration

Cell wall depended trace metal sequestration was considered as the best method of plants to escape from the toxicity of HMs (Macfie and Welbourn, 2000; Krzesłowska, 2011). The abundance of pectins, especially homogalactouronans (HGA) with functional groups such as -COOH, -OH and -SH in the cell wall determines the rate of accumulation of metal ion in cell wall (Davis et al., 2003). The affinity of pectin towards different metal ions is as follows: Al^{3+}> Pb^{2+}> Cu^{2+}> Co^{2+}> Ni^{2+}> Zn^{2+}> Cd^{2+}> Ca^{2+}, which indicates that Ca^{2+} in the cell wall can be easily replaced by the Cu^{2+}, Zn^{2+} and Cd^{2+} (Fritz, 2007). The 'copper moss' (*Scopelophila cataractae*), tolerates copper toxicity by binding most of the Cu to the pectin of the cell wall

(Konno et al., 2010). A well-known hyperaccumulator *Thlaspi caerulescens,* amass more than 50% of heavy metal in its cell wall (Salt et al., 1999). Most of the heavy metals distributed in rhizosphere are as cations, so plant cation exchange capacity and cell wall permeability are largely depended on heavy metal tolerance and this was proved using tolerant and sensitive varieties of wheat cultivars. The modified cell wall permeability of Al^{3+} was considered as a metal tolerant mechanism in wheat plants (Masion and Bertsch, 1997).

AM induced cell wall biosynthesis was observed in plants subjected to different biotic and abiotic stresses. Enhanced accumulation of phenolics and callose (components of cell wall) in mycorrhizal root cells was reported by Benhamou and Lafontaine (1995) under biotic stress. Moreover, the stimulation of cell wall apposition in mycorrhiza associated tissue was proved by Cordier et al. (1998). They observed this modification in *Phytophthora parasitica* infected tomato roots associated with *Glomus mossae*. The AM induced cell wall biosynthesis in host root facilitates metal detoxification, because of the increase in cell wall thickness, which increases the area for metal adsorption (Zhang et al., 2019). AM induced cell wall production augment the cell wall mediated metal sequestration in host cell and thus increases the metal accumulation potential of a hyperaccumulator.

2.6.2.4 Balanced plant mineral nutrition

Improved mineral absorption helps the plants to overcome HM toxicity. Zouari et al. (2016) reported that the external supply of mineral nutrients to plants under Cd stress reduced its toxic effects on plants. They explained the competition between Cd and mineral nutrients for the same transporters as the major reason of nutrient deficiency and it was overcome with optimal feeding of specific mineral nutrients. It was proved that AM can establish balanced nutrition to plants under different abiotic stresses like metal toxicity. Mycorrhizal mediated enhancement in phosphorus (P) absorption

was well explained in many relevant literatures (Campos et al., 2018). $LePt_1$, $StPt_3$ and $OsPt_{11}$ were the P transporters with enhanced function in tomato, potato and rice respectively when the arbuscule formation was established by the mycorrhiza in host cells (Liu et al., 2018). Liu et al. (2018) also found that mycorrhiza improves plant phosphorus level by inducing the overexperssion of *ZmPt9* (Pi transporter gene) in maize plants. Christie et al. (2004) reviewed AM involved improvement of plant growth in Zn contaminated soil due to higher uptake of P. Absorption of nitrogen, an essential element needed for the normal growth and development of plants was also enhanced during AM symbiosis (Nouri et al., 2014). This mutualistic symbiosis also improved the nutrition of potassium in plants (Garcia and Zimmermann, 2014). Mycorrhizae induced overexpression of *LiSultr12* gene, which encode specific protein involved in sulfate uptake, was reported in *Lotus japonicus* by Giovannetti et al. (2014). The non-mycorrhizal plants severely reduced the absorption of essential minerals under high concentration of toxic metal ions in soil, whereas AM mediated enhancement in mineral uptake under metal stress aid the plants to overcome the metal toxicity.

2.6.2.5 Non protein thiols

Major metal tolerance mechanism specific to a hyperaccumulator with phytoextraction potential is enhanced biosynthesis of non protein thiols and high metal translocation from root to shoot. Many metals show high affinities to thiols like glutathione (GSH), phytochelatins (PC) and metallothionein (MT). All these compounds have an important role in metal chelation in the cytoplasm, when plants hyper accumulate metals. Mycorrhizal mediated transcriptional induction of genes related to the biosynthesis of thiol compounds in host plants was reported by Pallara et al. (2013). The metal binding ligands in the plant cells facilitate HM detoxification and increase the tolerance level of plants towards toxic metal ions. In hyperaccumulator,

vacoular sequestration mainly depends on the PC and metal complexation and this helps to enhance its metal remediation potential (Hossain et al., 2012).

Glutathione (GSH)

GSH has an important role in metal localization and antioxidant potential of plants under HM stress. As a part of ascorbate-glutathione cycle, H_2O_2 will be converted into non-toxic forms (Miller et al., 2010). It was reported that GSH act as the precursor of phytochelatins (PC) by transferring a γ-Glu-Cys moiety from a donor to an acceptor molecule (Grill et al., 2007) catalysed by PC synthase or γ-Glu-Cys dipeptidyl transpeptidase (EC 2.3.2.15), which gets activated only in the presence of metal ions at *in-vitro* conditions. GSH mediated Zn tolerance was observed in bean leaves (Cuypers et al., 2001). Increased expression of the gene responsible for glutathione synthesis was observed in *Arabdiopsis* exposed to Cd and Cu stress (Xiang and Oliver, 1998). In Cd contaminated soil, leaves of *Solanum photeinocarpum* exihibited mycorrhizal mediated enhancement in GSH biosynthesis and metal tolerance (Tan et al., 2015). Garg and Kaur (2013) studied the Cd and Zn tolerance level of two genotypes (Sel 85N and P792) of *Cajanus cajan* associated with AM. They reported that AM fungi alleviated metal induced oxidative stress through the induced synthesis of GSH and based on this they concluded that one of the variety Sel 85N is a tolerant genotype. Many reports points towards the mycorrhizal mediated augmentation in GSH synthesis, which facilitate vacuolar sequestration of metals and thus increases the tolerance potential of the host.

Phytochelatins (PC)

In many plants, HM detoxification depends on the synthesis of cysteine rich polypeptides known as phytochelatins, which helps in the precipitation of metal ions in the vacuole (Cobbett, 2000). The biosynthesis pathway of

phytochelatins was studied using Cd sensitive *cad1* and *cad2* mutants of *Arapdiopsis thaliana* that was deficient in PC. PC inactivates toxic ions by making complexes with it. The inactive metal-PC complexes are further transported from cytosol to vacuole (Wu and Zhang, 2003). Inside the vacuole (low pH) it undergoes dissociation and the metals are further combined with acids (Clemens, 2006). It was reported that metal ions act as the inducer of PC biosynthesis and their chelation act as a signal of inhibiting the biosynthesis of the same (Loeffler et al., 1989).

Cu and Cd induced the formation of PC complexes in *Silene vulgaris* and these HM-PC complexes disappeared from the root cytoplasm, indicating transient nature of PC complexes (Leopold et al., 1999). It was proved that the 'disappeared HMs' was transported to vacuoles as PC associated complexes, and they get accumulated over there. Mycorrhizal mediated enhancement in PCs production was observed in the leaves of *Solanum photeinocarpum* under Cd stress (Tan et al., 2015). Andrade et al. (2010) concluded that AM induced PCs synthesis depended on the HM concentration imparted to the plants. They observed that the leaves of *Canavalia ensiformis* showed a reduction in PC production in low Cu level but it gets increased in higher Cu level as compared to non AM plant. Enhanced rate of PCs mediated Cd and Zn alleviation was observed in *Cajanus cajan* associated with *Glomus mossae* (Garg and Kaur, 2013). These findings signify the mycorrhizal induced accumulation of PCs which are involved in the metal detoxification and has the potential to enhance the HM accumulation and phytoremediation potential of host plants.

Metallothioneins (MTs)

Another important metal binding polypeptide, metallothioneins (MTs) was the first identified Cd binding proteins in mammalian tissues. MTs are also produced in plants when exposed to high concentrations of heavy metals

such as Cu, Zn and Cd. Plant MTs are generally classified into four groups, based on the cysteine residue arrangement (Grennan, 2011). AM mediated metal tolerance was observed in a transgenic tobacco bearing yeast MT gene combined with a polyhistidine cluster. AM associated roots under HM stress was found to overexpress specific genes responsible for the production of metallothioneins that increase the tolerance of plants to HM stress (Rivera-Becerril et al., 2005).

2.6.2.6 Antioxidative defense in host

The antioxidative defense system of a plant was highly active during HM stress (Kumar et al., 2009). The antioxidation system of plants comprised of metabolites like phenolics, ascorbate, glutathione, tocopherol *etc*. and enzymatic ROS scavengers such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPOX), ascorbate peroxidase (APX) and glutathione reductase (Asada, 1999; Michalak, 2006). The metal tolerance capacity of a hyperaccumulator depends on the activity of the antioxidative system. Many authors have discussed the efficiency of AM to induce the synthesis of antioxidants and enhance the activity of the same in various plants. For example, Garg and Aggarwal (2012) proved the stress alleviating potential of AM in genotypes of *Cajanus cajan* by up-regulating antioxidative activity. AM fungi decrease the production of ROS and malondialdehyde (MDA) under oxidative stress and it helps to ameliorate the toxic effects of HMs on host plants such as jack bean and *C. cajan* (Rabie, 2005a; Andrade et al., 2009; Garg and Kaur, 2013).

2.6.2.6.1 Antioxidant enzymes

Enzymatic antioxidants are essential to plants for the reduction of superoxides and metastable hydroperoxides formed in the plants under HM stress. Mycorrhizae mediated enhancement in the antioxidant enzymes

production in *Cajanus cajan* was reported by Garg and Kaur (2013). This was supporting the findings of Malekzadeh et al. (2007), who worked on tomato plants associated with *Glomus etunicatum* under copper stress. They observed elevation of APX and GPOX activities in mycorrhizal tomato shoot and root respectively when compared to nonmycorrhizal plants. Tan et al. (2015) investigated the effect of *Glomus versiforme* on antioxidant parameters of *Solanum photeinocarpum,* and they found that in Cd contaminated soil, leaves of *S. photeinocarpum* associated with AM, showed an improvement in CAT, GPX and APX activities which helped to avert the damages to biomolecules induced by ROS. Mycorrhiza induced enhancement in the activities of different antioxidant enzymes, increase the metal tolerance potential of plants during HM stress.

2.6.2.6.2 Non enzymatic antioxidants

Proline

Proline is an imino acid, which acts as a compatible solute and helps plants to overcome many abiotic stresses. Metal induced proline accumulation was observed in many plants (Sharma and Dietz, 2006; Andrade et al., 2009; Janeeshma and Puthur, 2020). Rai et al. (2004) proved the role of proline in HM stress alleviation by observing an increase in its accumulation in *Ocmium tenuiflorum* subjected to Cr stress. In general, leaves of AM associated plants like *Saccharum spontaneum* exhibited higher proline content as compared to non AM plants (Mirshad and Puthur, 2017). Mycorrhizal jack bean plants showed an augmentation in Cu induced proline accumulation, indicating the role of this amino acid in detoxification of metals (Andrade et al., 2010). Contradictory to this, Andrade and coworkers (2009) reported that mycorrhizal plants maintained lower proline content and other amino acids contents under elevated Zn stress and it drastically increased in non AM plants. This could be probably because the stress impacts were less in AM

plants, therefore the demand for the overproduction of proline was also simultaneously less.

Sugars

Keunen et al. (2013) reviewed the crucial role of soluble sugars such as disaccharides, raffinose family oligosaccharides and fructans in abiotic stress tolerance. Sugars have the capacity to scavenge OH^-, where normal enzymatic reduction of this free radical does not exist (Nishizawa et al., 2008). This ROS scavenging mechanism helps the plant to sustain in HM contaminated soil. There are reports regarding the enhancement of sugar synthesis in AM associated plants subjected to different kinds of abiotic stresses. Garg and Aggarwall (2012) reported increased accumulation of sugars in *Cajanus cajan* associated with *Glomus mossae* under Cd stress. The enhancement of glucose in plants under metal stress was the result of metal mediated inhibition of glucose oxidase (EC 1.1.3.4), an enzyme responsible for the oxidation of glucose (Chen et al., 2011). This AM induced enhancement of sugar helps a hyperaccumulator to sustain in a metal contaminated land.

Mycorrhizae mediated alterations in phenylpropanoids pathway

Phenylpropanoids are bioactive compounds, considered as secondary metabolites which can impart stress tolerance potential to plants (Michalak, 2006). The precursor of all phenylpropanoids is L-phenylalanine, an aromatic amino acid synthesized via the shikimate pathway. The core phenylpropanoid metabolism includes the formation of cinnamate and 4-coumaryl Co A from L-phenylalanine by the catalytic action of phenylalanine ammonia-lyases (PAL). Lignin, flavonols, flavanones, isoflavonoids, anthocyanins, tannin, benzoic, salicylic, caffeic, ferulic and sinapic acids are other important phenylpropanoids originating from cinnamate and 4-coumaryl Co-A. It is evident that most of these phenolic compounds have antioxidation potential

which can scavenge the ROS developed by the rapid ionic imbalance stimulated by excess metal ions. An enhancement in the biosynthesis of different phenolic compounds and the activity of different enzymes involved in the phenylpropanoid pathway was observed in plants under various metal stresses.

Metal induced accumulation of phenolics was reported in *Pinus sylvestris* (Schutzendubel and Polle, 2002), *Capsicum annuum* (Dıaz et al., 2001), *Verbascum thapsus* (Morina et al., 2010), *Lupinus luteus* (Izbianska et al., 2014) and Water lily (Lavid et al., 2001). Chlorogenic, p-coumaric, caffeic, ferulic and sinapic acids, lignin, six derivatives of benzoic acid, glycoside-bound phenolics, genistein, tannin, gallic acid and tannic acid derivatives were the major phenolic compounds elicited in response to metal toxicity (Lavid et al., 2001). The enhanced accumulation of chlorogenic acid under Cu and Cd toxicity in the root of *Matricaria chamomilla* is a clear indication of its antioxidant potential (Kováčik and Bačkor, 2008). Lignin, an important phenolic heteropolymer, accumulated in *Capsicum annuum*, *Glycine max*, *M. chamomilla*, and *Arabidopsis thaliana* (Dıaz et al., 2001; Kováčik and Bačkor, 2008; Lequeux et al., 2010; Pawlak-Sprada et al., 2011) subjected to metal stress and the accumulation of lignin helps to reduce the influx of metal ions and simultaneously prevent metal translocation to the shoot system. Tannin induced metal chelation was reported in waterlily subjected to Cr, Hg, Pb and Cd stresses (Lavid et al., 2001). Vitamin E (α-tocopherol) and anthocyanin were also elevated in response to Cd and Cu treatments in *Z. mays* and *A. thaliana* under heavy metal stress (Tanyolac et al., 2007; Collin et al., 2008).

PAL is a pivotal enzyme in the phenylpropanoid pathway, which was overexpressed in different plants during different metals toxicity. The correlation of PAL activity and transcription of a specific gene for its

synthesis was studied in *Glycine max* and *Lupinus luteus* under Cd and Pb stress by Pawlak-Sprada et al. (2011) and they confirmed an increase in the PAL activity at higher concentrations of metals. The protein profile of soybean cells also showed the enhanced production of another important enzyme in the phenylpropanoid pathway, chalcone synthase (CHS) under Cd stress (Sobkowiak and Deckert, 2006).

Mycorrhizal mediated alterations in the phenolic compounds was reported in *Allium cepa* (Grandmaison et al., 1993), *Ocimum basilicum* (Toussaint et al., 2007), *Arachis hypogaea* (Devi and Reddy, 2002), *Salvia officinalis* (Nell et al., 2009), date palm (Abohatem et al., 2011) and *Pteris vittata* (Leung et al., 2006). Toussaint et al. (2007) reported the efficiency of *G. caledonium* and *G. mosseae* to enhance the accumulation of rosmarinic and caffeic acids in the leaves of *O. basilicum*, which indicates the capability of mycorrhiza to alter the phytochemistry of shoot even though it is present only in the root system. Association of *G. intraradices* and *G. vesiforme* induced the production of ferrulic acid, p-coumaric acid and N-feruloyltyramine in roots of onion and these antimicrobial compounds attributes to the potential of the plant towards prevention of pathogenic attack to mycorrhizae associated roots (Grandmaison et al., 1993). Similarly, development of different phenolics compounds in the root of *A. hypogaea* was observed as a result of AM association and these compounds were caffeic acid, protocatechuic acid, chlorogenic acid, *p*-coumaric acid, hydroxybenzoic acid, ferulic acid and vanillic acid (Devi and Reddy, 2002). Shutzendubel and Polle (2002) proposed that the phenolic compounds increases due to the mycorrhizal association and can play a crucial role in cell wall rigidification. Moreover, mycorrhizae could also influence the essential oil composition of leaves of *Origanum* sp (Khaosaad et al., 2006), *O. basilicum* (Copetta et al., 2006) and *Mentha arvensis* (Freitas et al., 2004). Metal induced up-regulation in the expression of phenylalanine ammonia-lyases and chalcone synthase

genes of *Trifolium repense* was related to AM colonization which ultimately leads to phenolic accumulation in AM associated roots (Zhang et al., 2013).

An increase in phenolics accumulation was reported in mycorrhizae associated *Solanum lycopersicum* under Cd stress and this could stimulate the antioxidant defence system of plants, which finally leads to alleviation of Cd stress effects (Hashem et al., 2016a). Even though there are difficulties to maintain a pure culture of mycorrhiza, *in vitro* studies were also carried out to examine the rate of phenolic compounds accumulation during HM stress. Khade and Adholeya (2008) clearly analyzed the effect of HM on the mycorrhizal symbiosis with the help of *in vitro* culture of Ri-TDNA (transfer DNA of root hair inducing plasmid) transformed roots of *Daucus carota*. They focused on the total phenol production and exudation of the same into the culture medium. When AM inoculated Ri-T-DNA transformed roots were subjected to Pb stress, the total phenol content increased in the root but decreases the exudation rate into the medium. Mycorrhizal association modified the synthesis of soluble and cell wall bound phenolics under metal toxicity. Mycorrhiza induced variation in hydroxycinnamates was analyzed in *Cichorium intybus* cultivated in metal polluted lands and the results revealed that metal toxicity alone did not stimulate the increase of hydroxycinnamates (Rozpadek et al., 2014). Mycorrhizal plants secreted salicylic acid, an important signaling molecule in maize plants under Cr stress, which was not detected in non AM plants under Cr stress (Gomathy et al., 2011).

Rhizophagus irregularis (a well-known AM with metal tolerance potential) association alleviated Cd stress in *Medicago truncatula* with a modification in flavonoids (Aloui et al., 2012). Accumulation of some isoflavonoids and their derivates; formononetin, malonylononin, medicarpin 3-O-b-(60-malonylglucoside), medicarpin and coumestrol were observed in Cd treated plants, but AM association helps the plants to avoid Cd

translocation and thereby reduce flavonoid accumulation. This can be correlated with the downregulation of chalcone reductase synthesizing gene in *R. irregularis*-colonized roots compared to noninfected roots of *M. truncatula* (Aloui et al., 2012; Aloui et al., 2009). Similarly, Schutzendubel and Pollen (2002) proposed that mycorrhization can reduce the oxidative stress induced by Cd in the roots of *Paxillus-Pinus* and it was proved based on the reduced accumulation of phenolics in mycorrhizal roots as compared to non mycorrhizal roots. All these reports indicate the potential of accumulated phenolics, as a result of AM association in sustaining the plant in a metal contaminated environment.

Generally, the AM association enhances major metal tolerance mechanisms of plants. Therefore, attempts have been made to compare and evaluate the direct and indirect influences of AM association in heavy metal tolerance. Information with regard to the role and mechanisms of AM in enhancing the phytoremediation potential of plants can help to develop strategies for further exploitation of AM fungus for this purpose.

MATERIALS AND METHODS

3.1 Materials

3.1.1 Collection of seeds

Oryza sativa L. and *Zea mays* L. belong to the family Poaceae. *Oryza sativa* (variety Varsha) seeds were collected from Regional Agricultural Research Station (RARS) of Kerala Agricultural University, Pattambi, Kerala, India and *Zea mays* (variety CoHM 6) seeds were collected from Centre for Plant Breeding and Genetics, Department of Millets, Tamil Nadu Agriculture University (TNAU), Coimbatore, India.

3.1.2 Collection of AM inoculum

Inocula of *Claroideoglomus claroideum* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler (=*Glomus claroideum* (N.C. Schenck & G.S. Sm.)) and *Claroideoglomus etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler (=*Glomus etunicatum* (W.N. Becker & Gerd.)) were procured from Centre for Mycorrhizal Culture Collection (CMCC), The Energy and Resources Institute (TERI), New Delhi.

3.1.3 Chemicals

Analytical (AR) and guaranteed (GR) grade chemicals were purchased from Merck, Himedia, Qualigens and SRL companies. Riboflavin, glutaraldehyde, bovine serum albumin (BSA), methyl viologen, 3- (3, 4-dichlorophenyl)-1,1-dimethyl urea (DCMU), sodium azide and L-ascorbate were purchased from Sigma Aldrich Co., USA.

3.2 Analysis of soil physicochemical properties

3.2.1 Soil particle-size determination

A small quantity of soil (15 g) was collected for the analysis of soil texture and 0.053 mm sieve was used to separate the sand fractions. The silt and clay solution was prepared in 250 mL distilled water and stirred thoroughly to suspend all the particles. The suspension was allowed to settle undisturbed at room temperature (18–24ºC) for a sedimentation period of 6 h. After the sedimentation, the suspended clay fraction was decanted from the settled silt particles and discarded. The settled silt and sand fraction was then dried at 105ºC until it attained constant weight. The soil sand% and silt% were calculated based on their respective fractions in the original sample mass,

$$\text{Sand \%} = \frac{\text{Oven dried sand mass}}{\text{Original soil mass}} \times 100$$

$$\text{Silt \%} = \frac{\text{Oven dried silt mass}}{\text{Original soil mass}} \times 100$$

The clay % was determined by calculating the difference between the original sample mass and the sum of the sand % and silt %,

$$\text{Clay \%} = 100 - (\text{Sand \%} + \text{Silt \%})$$

3.2.2 Estimation of phosphorus content in soil

Available phosphorus content was estimated by Bray's method No. 1 (Bray and Kurtz, 1945). Five grams of air-dried soil was mixed with 50 mL of Bray and Kurtz extracting solution (0.03 N NH_4F in 0.025 N HCl). An aliquot (5 mL) of the extract and made upto 20 mL with distilled water. To it 4 mL Murphy-Riley colour developing solution (a solution containing 250 mL of 2.5 M H_2SO_4, 75 mL ammonium molybdate solution, 50 mL ascorbic acid

solution, 25 mL of antimony potassium tartrate solution and 100 mL of distilled water) was added. After 15 min, the intensity of blue colour was measured at 730 nm.

3.2.3 Estimation of potassium content in soil

The potassium content in the soil was estimated using flame photometer (Bhattacharjee and Sharma, 2011). Five grams of air-dried soil was taken in a centrifuge tube and 25 mL of 1 N ammonium acetate solution was added, shaken well and centrifuged at 2,000 rpm for 10 min. The supernatant was collected and fed in the flame photometer.

3.2.4 Estimation of nitrogen content in soil

Nitrogen content of soil sample was measured with a Kelplus nitrogen estimation system (Pelican, Classic DX, Mumbai, India) by following the method of Zhang et al. (2016a). Firstly, 2.0 g air-dried soil powder was mixed with 6.2 g $K_2SO_4/CuSO_4.5H_2O$ catalyst (30:1), then 20 mL H_2SO_4 was added into the mixture and the mixture was digested at 42ºC for 1.5 h. At last, the nitrogen estimation system was used to distill the cooling liquid and assay nitrogen concentrations.

3.2.5 Estimation of Mg, Fe, Zn, and Ca in soil

Soil samples were prepared according to the method of Allan (1969). Soil sample was dried at 60ºC in a hot air oven. Known weights of the dried samples were digested using a mixture of nitric acid and perchloric acid in the ratio of 10:4 until the solution became colourless using Kjeldahl's flask heated on a heating mantle. This digest was filtered and made up to 50 mL using double distilled water. Atomic absorption spectrophotometer (GBC scientific equipment Ltd., GBC932 Plus-3000, Braeside VIC 3195, Australia)

was used for the estimation of Mg, Fe, Zn, and Ca present in the digested samples.

3.2.6 Estimation of organic carbon

Colorimetric method was used to estimate the organic carbon content of the soil (Datta et al., 1962). One gram soil sample was mixed with 10 mL of 1 N potassium dichromate and 20 mL conc. sulphuric acid, swirled and kept for 30 min on an asbestos sheet. The content was centrifuged to a clear state. The green colour of the supernatant was read using colorimeter at 660 nm. Anhydrous sucrose was used as the standard.

3.2.7 Estimation of soil protein content

Total glomalin related soil protein were extracted by the procedure of Wright and Upadhyaya (1998). One gram of air dried soil was placed in a 50 mL centrifuge tube and mixed with 8 mL of 50 mM sodium citrate buffer (pH 8), and then these samples were autoclaved for 30 min. Further, these samples were centrifuged in 5000 rpm for 15 min at 4ºC and the supernatant was poured off and the pellet was retained. Samples were sequentially extracted by adding an additional 8 mL of sodium citrate buffer to the soil pellet. Extractions was continued until the supernatant turned to a pale straw color, and finally the extracts were pooled. Glomalin related soil protein was estimated in the pooled supernatant by the Bradford assay with bovine serum albumin as standard.

3.2.8 Soil pH

For determining the soil pH, soil suspension was prepared by thoroughly mixing one gram of soil with 25 mL of distilled water (1:25). This mixture was kept undisturbed then filtered. The pH of the filtrate was measured using a pH meter (Eutech, PCSTEST35, Vernon Hills, USA).

3.3 Sterilization of the soil

The soil was solarized for avoiding the contamination by the protocol of Raj and Sharma (2009)**.** For the purpose of getting maximum penetration of sunlight, the soil was spread on a clean ground. The better conductance of heat was achieved by watering the soil. A clear thin polythene film was spread tightly over the soil to avoid air contact and this process was continued for 4 to 6 weeks.

3.4 Multiplication and preparation of the inoculum

For the multiplication of *C. claroideum* and *C. etunicatum* spores, pot culture method was adopted and *Z. mays* was selected as the host. For maintaining the isolated cultures of *C. claroideum* and *C. etunicatum*, polythene bags (18×13 cm) were filled with 1 kg of sterilized soil (soil:sand in 1:1) and the soil was inoculated with 1 g of each inocula procured from CMCC prior to sowing. The surface sterilized (with 0.1% $HgC1_2$ (w/v) solution for 5 min) Z. *mays* seeds were sown in each pot at 8 cm depth. After 2 months of growth, when the AM fungus colonization level was 100%, shoots of *Z. mays* were cut off and the remaining root parts were kept in the pot. The roots along with the soil in the pot was taken as the AM inoculum and it contained spores, mycelium and root fragments. Polythene bags were filled with 1 kg of solarized soil were used for this study. Forty grams of AM inoculum, as described above, containing roots and spores was added to the surface of the solarized soil in polythene bags. The AM inoculum added to the soil was mixed well with the soil using a sterile glass rod. After this, three seeds of *O. sativa* and *Z. mays* were sowed in each soil containing polythene cover. After 20 d, the roots of these plants were analyzed to confirm AM colonization. The plants after 45 d of growth were exposed to Cd and Zn toxicity.

3.5 Root colonization analysis

Roots samples were washed thoroughly in distilled water and segments into 1 cm root bits. Then placed in beaker contain 10% KOH and heated to 90 °C for 15-30 min. After the removal of excess KOH, the roots were immersed in H_2O_2 (3%) for 5-10 min. Further, the samples were treated with 5 N HCl for 2-3 min. The root segments were stained with 0.01% of trypan blue in lacto glycerol for 24 h at room temperature. Lacto glycerol was used to wash off the excess stain absorbed by the root segments (Philips and Hayman, 1970). The stained root samples were observed under microscope (Olympus CH 20i, Tokyo, Japan) for examining the association of AM fungus. The percentage of mycorrhizal colonization of was assessed using the following formula,

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

3.6 Determination of optimal quantity of AM inoculum

For determining effective concentration of inoculum, *O. sativa* as well as *Z. mays* were inoculated with 10, 20, 30, 40 and 50 g of *C. claroideum* and *C. etunicatum* inocula and the development of hyphal density were determined on 45 d of inoculation.

3.7 Isolation and germination analysis of spores

Zea mays plants which attained 45 d of growth was inoculated separately with *C. claroideum* and *C. etunicatum* and was treated with 40 mL of solutions containing 1.95 g Zn kg^{-1} soil as $ZnSO_4$ and 0.45 g Cd kg^{-1} soil as $CdCl_2$. The spores of *C. claroideum* and *C. etunicatum* isolated from the rhizosphere soil was collected in 2 d intervals (2, 4, 6 d) of HM treatment by wet sieving and decanting method described by Gerdeman and Nicolson (1963). For this, twenty grams of soil was suspended in 1 L tap water, stirred,

and filtered through four sieves with different pore size (45, 106, 250 and 355 μm). Materials collected from each sieves were collected to petri dishes for the observation of AM spores and sporocarps under a stereo microscope. Further, spore germination was evaluated using compound microscope (Olympus, CH 20i, Tokyo, Japan).

3.8 Determination of stress imparting concentrations of Cd and Zn

Zea mays and *Oryza sativa* were kept in polyhouse maintained at 60 ± 2 % relative humidity, 25 ± 5 °C temperature and 12 h day light ranging from 28 to 600 μmol/m^2/s (28 ± 4: 600 ± 50: 51 ± 10 μmol/m^2/s at 6 am: 12 am: 6 pm respectively). After 45 d of growth, plants were treated with 40 mL (field capacity of the soil) solutions of $CdCl_2$ and $ZnSO_4$. For the selection of stress imparting concentrations of Cd and Zn, plants were exposed to different concentrations of $CdCl_2$ (0, 0.225, 0.45, and 0.675 g Cd kg^{-1} soil) and $ZnSO_4$ (0.0, 0.65, 1.30, 1.95, and 2.62 g Zn kg^{-1} soil) and the preliminary analysis was conducted at 4 d intervals (0, 4, 8, and 12 d). Moisture content %, total chlorophyll and malondialdehyde (MDA) content were the parameters studied for the selection of stress imparting concentration. The second lower leaf of *O. sativa* and *Z. mays* plants were taken for various analysis.

3.9 Experimental design

Surface sterilized *Z. mays* and *O. sativa* seeds (3 nos) were placed at 8 cm below the sterilized soil filled in polythene bags (18×13 cm). The physicochemical characteristics of this soil were determined as; 72.50% sand, 6.25% silt, 21.25% clay, pH 6.8, organic carbon 1.26%, 6200 mg kg^{-1} N, 5160 mg kg^{-1} K, 1265 mg kg^{-1} P, 1625 mg kg^{-1} Ca, 1123 mg kg^{-1} Mg, 90.6 mg kg^{-1} Fe, and 23 mg kg^{-1} Zn. Two set of plants were maintained for the experiment, one with AM inoculation and the other without AM inoculation. For AM inoculation 40 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 ± 5ºC temperature and 12 h day light ranging from 28 to 600 µmol/m^2/s. Plants were initially watered with distilled water and fertilized with 25 mL of quarter-strength modified Hoagland solution (The Hoagland solution was prepared by avoiding $ZnSO_4$. $7H_2O$). As the plants grew up (to 45 d), just one plant was maintained in a pot cover and all others were uprooted carefully and discarded. The composition of Hoagland solution is detailed in table 2. 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 $CdCl_2$. Various analysis were conducted in 4 d intervals (0, 4, 8, and 12 d) and the second lower leaf and the roots of the plants were taken for various analysis.

Table 2: Composition of modified Hoagland solution used in the present study.

	Compounds	Molecular weight	Concentration of stock solution	Volume of stock solution/L of final solution
		(g mol^{-1})	(mM)	(mL)
Micronutrients	KNO_3	101.10	1000	6.0
	$Ca(NO_3)_2.4H_2O$	236.16	1000	4.0
	$NH_4H_2PO_4$	115.08	1000	2.0
	$MgSO_4.7H_2O$	246.48	1000	1.0
Micronutrients	KCl	74.55	25	2.0
	H_3BO_3	61.83	12.5	
	$MnSO_4.H_2O$	169.01	1.0	
	$CuSO_4.5H_2O$	249.68	0.25	
	H_2MoO_4	161.97	0.25	
	NaFeEDTA	558.50	53.7	0.3

3.10 Physiological parameters

3.10.1 Fresh weight (FW) and dry weight (DW)

Fresh weight of *Z. mays* and *O. sativa* plants was directly measured using an electronic weighing balance. For dry weight measurements, the weighed samples were dried in a hot air oven at 100^{o}C for 1 h and later the temperature was set at 60^{o}C, in a hot air oven, until the weight attained a constant value.

3.10.2 Tissue moisture content %

The moisture content of leaves and roots of *O. sativa* and *Z. mays* plants was determined by measuring the fresh and dry weights of the leaves and roots (Lokhande et al., 2011). Tissue moisture content percentage was calculated using the following equation.

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

3.10.3 Root volume

The root volume of *Z. mays* and *O. sativa* plants was measured according to the protocol of Rahul et al. (2019). A glass beaker was taken for measuring the root volume, which was filled with water up to the brim and then the roots were completely immersed in the beaker allowing the water to overflow and then the roots were removed from the beaker. The water over flown was measured by refilling the glass beaker up to brim with the help of a measuring cylinder. The root volume reading was recorded in cm^3.

3.10.4 Determination of cell viability

The loss of cell viability in the roots of metal treated plants was evaluated by the modified method of Šimonovičová et al. (2004), using Evans blue stain. Roots were stained in 0.25% (v/v) aqueous solution of Evans blue

for 15 min at room temperature. The stained roots were washed three times with distilled water, for 10 min each. Root tips (5 mm) were excised and soaked in 50% (v/v) methanol along with 1% (w/v) sodium dodecyl sulphate (SDS) at 60°C for 1 h. Optical density was measured spectrophotometrically at 600 nm.

3.10.5 Leaf pigment composition

Total chlorophyll contents were estimated according to the method of Arnon (1949) whereas the carotenoid content was analysed as per the protocol of Lichtenthaler and Wellburn (1983). Two hundred mg fresh leaf sample was weighed using electronic balance and crushed in 80% acetone using mortar and pestle. The homogenate was centrifuged at 5000 rpm for 10 min at 4°C and the supernatant was collected. Re-extraction was performed with 80% acetone until the pellet became colourless. The absorbance was read at 663, 646, 750, and 470 nm. Total chlorophyll (Chl *a*+*b*) and carotenoids present in the extract was calculated as microgram chlorophyll and carotenoids per gram fresh weight using the following formula,

$$\text{Chlorophyll } a + b = \frac{20.12\,(A_{646} - A_{750}) + 8.02\,(A_{663} - A_{750})}{\text{Fresh weight of the sample}} \times \text{volume}$$

$$\text{Carotenoids} = \frac{1000\,(A_{470}) + 3.27\,(\text{Chl } a - \text{Chl } b)}{\text{Fresh weight of the sample} \times 229} \times \text{volume}$$

Where,

$$\text{Chlorophyll} = \frac{12.69(A_{663} - A_{750}) - 2.69(A_{646} - A_{750})}{\text{Fresh weight of the sample}} \times \text{volume}$$

$$\text{Chlorophyll } b = \frac{22.9(A_{646} - A_{750}) - 4.68(A_{663} - A_{750})}{\text{Fresh weight of the sample}} \times \text{volume}$$

3.10.6 Chlorophyll (Chl) *a* fluorescence analysis

Chl *a* fluorescence analysis was performed using Plant Efficiency Analyzer (Hansatech Handy PEA, Norfolk, UK), which is a portable fluorometer having high resolutions (Strasser et al. 2004). All measurements were performed on the upper surfaces of the second lower leaves of *O. sativa* and *Z. mays* on 8 d of stress, after dark adaptation for 20 min using the light exclusion clips and later by illuminating with a continuous red light of high intensity (1500 μmol m^{-2} s^{-1}). All the measurements were recorded up to 1 second and the average values from 30 measurements recorded on the second lower leaves of the plant for each treatment were used for the analysis. The radar plot and energy pipeline model was deduced using the Biolyzer HP3 software (Chl *a* fluorescence analyzing program by Bioenergetics Laboratory, University of Geneva, Switzerland). All the parameters selected for the analysis are represented in table 3.

Table 3. Explanations of chlorophyll *a* fluorescence parameters used in the present study.

Parameters	Description
Phases in induction curve	
O=F_O	Minimal fluorescence/ first step of chl *a* fluorescence transient
I=F_I	Intermediate step in the chl *a* fluorescence transient at 2 ms
J=F_J	Intermediate step in the chl *a* fluorescence transient at 30ms
P=F_P=F_M	Maximal fluorescence level/ final step of chl *a* fluorescence transient
K	Intermediate step in the chl *a* fluorescence transient at 0.3 ms
OJ-phase	It represents the reduction of the acceptor side of PSII
JI-phase	It represents the reduction of the PQ (Plastoquinone) pool

IP-phase	It represents the reduction of the acceptor side PS I
Area	Area above tha fluorescnce induction curve
t_{FM}	Time taken to reach F_M
Other JIP parameters	
F_V	Maximal variable fluorescencce (F_M-F_O)
F_V/F_M	It represent maximum quantum yield of PSII
F_V/F_O	It represents the maximum efficiency of water splitting complex
S_M	It represents multiple turnover of Q_A reductions
S_M/ t_{FM}	It representing the average redox state of Q_A in the time span from 0 to t_{FM}
N	Turn over number of Q_A indicates the number of times Q_A was reduced from 0 to t_{FM}
SFI $_{ABS}$	An indicator of PSII structure and functioning
V_J	Relative variable fluorescence at phase J of the fluorescence induction curve
V_I	Relative variable fluorescence at phase I of the fluorescence induction curve
PI $_{ABS}$	Performance index of PSII on absorption basis
PI $_{TOTAL}$	Performance index of electron flux to the final PSI electron acceptors
10RC/abs	Absorption per RC
RC/CS_M	density of active PS II reaction centers per cross section
DF_{ABS}	PSII-relative driving force index on an absorption basis
Kn	Non-photochemical de-excitation rate constant
Kp	Photochemical de-excitation rate constant
Yield parameters	
φPo	Maximum quantum yield of primary PSII photochemistry (at t = 0)
φ (Do)	Quantum yield of energy dissipation
φ(Eo)	Quantum yield (at t = 0) for electron transport from QA^- to plastoquinone
Ψo	Probability (at t = 0) that a trapped exciton moves an electron into the electron transport chain beyond QA
ϒRC	Probability that PSII chlorophyll molecule functions as RC
δ(Ro)	Efficiency/probability (at t = 0) with which an electron from the intersystem carriers moves to

	reduce end electron acceptors at the PSI acceptor side
Specific energy flux	
ABS/RC	Absorption flux per RC corresponding directly to its apparent antenna size
TRo/RC	Trapping flux leading to QA reduction per RC at t = 0
ETo/RC	Electron transport flux from QA^- to plastoquinone per RC at t = 0
DIo/RC	Dissipated energy flux per RC at the initial moment of the measurement, i.e., at t = 0
Phenomenological energy flux	
ABS/CSm	Absorption of energy per excited cross-section (CS) approximated by F_M
TRo/CSm	Excitation energy flux trapped by PSII of a photosynthesizing sample cross-section (CS) approximated by F_M
ETo/CSm	Electron flux transported by PSII of a photosynthesizing sample cross-section (CS) approximated by F_M
DIo/CSm	Heat dissipation of excitation energy by PSII of a photosynthesizing sample cross-section (CS) approximated by F_M

3.10.7 Photosystem I and II activities

Thylakoids from leaves were isolated (Puthur, 2000; Janeeshma et al., 2021b). The photochemical activities of the isolated thylakoids were assayed polarographically with a Clark-type oxygen electrode (Hansatech DW1/AD, Norflok, UK) which was connected to a digital control box (Hansatech OXYG1, Norflok, UK) at 4°C. The light dependent O_2 uptake/evolution was measured by irradiating the sample with saturating intensity of white light (1800 µmol photons $m^{-2}s^{-1}$), provided by a 100W halogen lamp (Hansatech LS2, Norflok, UK). The activities of and PSI and PSII was expressed in terms of µmol of O_2 consumed (PSI)/evolved (PSII) min^{-1} mg^{-1} chlorophyll.

3.10.7.1 Isolation of thylakoid membranes

Five hundred milligram of fresh leaf tissue was homogenized in 6 mL of ice-cold isolation buffer (pH 7.8) containing 400 mM sucrose, 20 mM tricine, and 10 mM NaCl with a chilled mortar and pestle. The resulting homogenate was filtered through a cheesecloth, further the filtrate was centrifuged at 5000 rpm for 6 min at 4°C. The pellet was suspended in 250 µL suspension buffer (pH 7.5) containing 10 mM NaCl, 20 mM HEPES [N-(2-Hydroxyethyl) piperazine-N-(2-Ethanesulphonic acid)], 100 mM sucrose and 2 mM $MgCl_2$ and it was transferred to a clean test tube. This thylakoid suspension was stored at 4°C.

3.10.7.2 Estimation of chlorophyll content of thylakoid membranes

Chlorophyll content of the thylakoid samples was estimated (Arnon, 1949; Janeeshma et al., 2021b). according to the method of Arnon (1949). 20 µL of the thylakoid suspension was added to the test tube containing 3 mL of 80% acetone (v/v). The contents of the tubes were mixed thoroughly using a vortex mixer and the homogenate was centrifuged at 5000 rpm for 5 min and the supernatant was collected. The absorbance was measured at 645, 663 and 750 nm against the solvent blank (80% acetone). The total chlorophyll content of the thylakoids was calculated using the following equation:

$$\text{Total chlorophyll} = 20.12\ (A_{646}\text{-}A_{750}) + 8.02\ (A_{663}\text{-}A_{750}) \times \text{Dilution factor}$$

3.10.7.3 Analysis of thylakoid electron transport activities

PSI activity was measured in terms of oxygen consumption by using artificial electron donor 2, 6-dichlorophenolindophenol (DCPIP) and methyl viologen (MV) as exogenous electron acceptor. The reaction mixture contained reaction buffer, ascorbate (600 µM), MV (500 µM), sodium azide (NaN_3) (1 mM), 3- (3, 4-dichlorophenyl)-1, 1-dimethyl urea (DCMU) (5 µM),

DCPIP (0.1 mM). Chloroplast suspension equivalent to 10 μg chlorophyll was added and the volume was made up to 2 mL with reaction buffer. PSII activity was measured in terms of oxygen evolution by using 500 μM phenyl-p-Benzoquinone (pBQ) as artificial electron acceptor.

The light dependent electron transport in the thylakoid membrane was blocked in certain steps, by artificial electron acceptors and donors for estimating the PSI and PSII activities based on the O_2 uptake/evolution from/into the medium. For measuring PSI activity, PSII activity was blocked initially by adding DCMU to the medium. Electron transport to PSI was maintained by artificial electron donors, ascorbate and DCPIP in the medium, where ascorbate acted as reductant by donating electrons to DCPIP and further the electrons supplied by DCPIP to plastocyanin were transferred to PSI. Electrons from PSI are bypassed to an artificial electron acceptor, MV in the reaction mixture. MV reacts with oxygen molecules in the medium and produce H_2O_2. Further the dissociation of H_2O_2 to form oxygen and H_2O by the action of catalase is arrested by NaN_3 added in the reaction mixture. Thus the oxygen consumption by activity of PSI alone is measured by oxygen electrode system.

To measure the PSII activity, artificial electron acceptor pBQ was added in the medium and it will scavenge the electrons from plastoquinone. The transfer of electron from plastoquinone to cytochrome is terminated and so the activity of PSII alone was measured. Splitting of water for transferring of electrons to PSII result in evolution of oxygen molecules in the medium and it was measured by the oxygen electrode system.

3.10.8 Leaf micromorphological characters

Micromorphology of leaves were evaluated using scanning electron microscope (SEM) on 8 d of Cd and Zn stresses. The leaf cuttings of different treatments were fixed in 2.5% glutaraldehyde, prepared in 0.1 M sodium cacodylate buffer (pH 7.2) for 5 min. Fixed specimens were rinsed twice with double distilled water and dehydrated by passing through an ascending acetone concentration series. Dried specimens were mounted on to grooves cut on aluminium stubs using double side adhesive conducting carbon tapes to expose the sections. After gold-palladium coating, photomicrographs of the specimens were taken using the photographic attachment of the Field Emission Scanning Electron Microscope (Zeiss Gemini 300, Berlin, Germany).

3.11 Biochemical parameters

3.11.1 ROS types

3.11.1.1 Superoxide ($O_2^{\cdot-}$) content

Superoxide content in the leaves and roots of *Z. mays* and *O. sativa* was estimated as described by Doke (1983).

***Extraction*:** Two hundred milligram of leaf and root samples were cut into 1×1 mm size and immersed in 0.01 M potassium phosphate buffer (pH 7.8) containing 0.05% nitroblue tetrazolium (NBT) and 10 mM NaN_3.

***Estimation*:** The mixture was kept in water bath (85°C) for 15 min. After incubation, the mixture was quickly transferred to ice bath for cooling, and absorbance of the mixture was measured at 580 nm. Sodium nitrate ($NaNO_2$) was used as the standard.

3.11.1.2 Hydrogen peroxide content

Hydrogen peroxide content in the leaves and roots of *Z. mays* and *O. sativa* was estimated as described by Junglee et al. (2014).

***Extraction*:** Two hundred mg of tissue homogenized in 5 mL of 0.1% ice cold trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000 rpm for 15 minutes. The supernatant was collected and used for the estimation of hydrogen peroxide.

***Estimation*:** Five hundred microlitre of the supernatant was mixed with 0.5 mL of potassium phosphate buffer (pH 7), to which 1 mL of 1 M potassium iodide was added. The absorbance of the mixture was measured at 390 nm. Hydrogen peroxide was used as the standard.

3.11.2 Lipid peroxidation

The methodology of Heath and Packer (1968) was adopted to estimate the malondialdehyde content (MDA) in the leaves and roots of *Z. mays* and *O. sativa*.

Extraction: Two hundred milligram of the leaves and roots was weighed in duplicate and homogenized in 5 mL of 5% TCA. The homogenate was centrifuged at 12,000 rpm for 15 min at room temperature. The supernatant was collected and used for the estimation of MDA.

Estimation: Two millilitre of the supernatant was mixed with an equal aliquot of 0.5% of thiobarbituric acid (TBA) in 20% TCA. The solution was heated at 95°C for 24 min, cooled and then centrifuged at 3000 rpm for 2 min. The absorbance of the supernatant was measured at 532 and 600 nm against reagent blank using a UV-VIS spectrophotometer (Systronics 2201, Ahmadabad, India). The absorbance value at 532 nm was corrected for non-specific turbidity by subtracting absorbance value at 600 nm; and then the

MDA content was calculated using its extinction coefficient of 155 mM^{-1} cm^{-1}.

3.11.3 Membrane stability index (MSI)

Membrane stability index (MSI) was estimated as described by Sairam et al. (1997). One hundred milligram of fresh tissue was cut into 10 mm^2 sized segments and placed in tubes containing 5 mL of distilled water in two sets. One set was kept at 40°C for 30 min and electric conductivity (C_1) was measured by conductivity electrode (PCSTEST35, Eutech, Vernon Hills, USA).

Another set was kept in boiling water bath (100°C) for 15 min and its electric conductivity (C_2) was also measured. The MSI was calculated as,

$$MSI = [1 - (C_1/C_2)] \times 100$$

3.11.4 Electrolyte leakage (EL%)

Electrolyte leakage (EL%) was estimated as described by Lutts et al. (1996) with modifications. Two hundred milligram of fresh tissue was cut into 10 mm^2 sized segments and placed in tubes containing 25 mL of distilled water and was kept at 4°C for 24 h and then brought to room temperature and electrical conductivity was measured (EC_1). Further the tissue was autoclaved at 120°C for 15 min and electrical conductivity (EC_2) was measured again by conductivity electrode (Eutech PCSTEST35, Vernon Hills, USA). The EL% was calculated as,

$$EL\% = (EC_1/EC_2) \times 100$$

3.11.5 Primary metabolites

3.11.5.1 Soluble protein

Soluble protein content in the leaves and roots of *Z. mays* and *O. sativa* was estimated using Coomassie brilliant blue dye according the method of Bradford (1976).

Extraction: Five hundred milligram of leaf tissue was homogenized in 5 mL of phosphate buffer (pH 7) using pre-chilled glass mortar and pestle. Further, it was centrifuged at 5000 rpm for 10 min at 4°C and the supernatant was collected.

Estimation: For estimating soluble protein in the supernatant, method of Bradford (1976) was followed. One hundred microlitres of supernatant was pipetted out to test tubes and was made up to 1 mL using distilled water. To this 5 mL of Coomassie brilliant blue dye was added and mixed well. It was kept undisturbed for 10 min and the absorbance was read at 595 nm. BSA was used as standard and the concentration of protein was calculated from standard curve.

Preparation of dye: Coomassie brilliant blue dye was prepared by dissolving 100 mg Coomassie brilliant blue G 250 in 50 mL of 5% ethanol. Two hundred microlitres of concentrated orthophosphoric acid (85%) was added into this and it was made up to 1000 mL. It was then filtered through two layered Whatman No. 1 filter paper. Dark condition was maintained throughout and the dye was stored in amber coloured bottles.

3.11.5.2 Total soluble sugar

The total soluble sugar in the leaves and roots of *Z. mays* and *O. sativa* was estimated using the method proposed by Dubois et al. (1956).

Extraction: Two hundred milligram tissue was homogenized in 80% ethyl alcohol (v/v) using a clean glass mortar and pestle. The homogenate was boiled for 1h in a water bath at 100°C. After cooling, it was centrifuged at 8000 rpm for 10 min at 4°C and the supernatant was dried in a hot air oven at 60°C.

Estimation: From the eluted sample, a 100 µL of aliquot was taken in the test

tube and made upto 1 mL using distilled water. To this, 0.1 mL of 5% (w/v) phenol was added and mixed well. Five mL of the concentrated sulphuric acid was added to this. After cooling, the absorbance was measured at 490 nm using a spectrophotometer (Genesys 20). D-glucose was used as the standard.

3.11.5.3 Total free amino acids

Total free amino acids in the leaves and roots of *Z. mays* and *O. sativa* was determined according to the method of Moore and Stein (1948).

Extraction*:* Two hundred milligrams of fresh samples was homogenized in a clean mortar and pestle with 80% (v/v) ethanol. The extract was centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was made up to 10 mL with 80% ethanol.

Estimation: One hundred microlitre of the sample was mixed with 1 mL of ninhydrin reagent in a test tube. Tubes were kept in boiling water bath for 20 min. After cooling, 5 mL of diluent (water and n-propanol in 1:1 ratio) was added to it. This mixture was incubated at room temperature for 15 min and the absorbance was read at 570 nm using a UV-VIS Spectrophotometer (Systronics 2201, Ahmadabad, India) against a reagent blank. Standard curve was plotted by using L-leucine.

***Preparation of the reagent*:** Reagent solution was prepared by dissolving 20 g of ninhydrin and 3 g of hydrindantin in 750 mL of methyl cellosolve. The solution was stirred carefully to avoid air bubbles in the solution. To this solution, 250 mL of sodium acetate buffer (pH 5.5) was added and the resulting reagent solution was immediately transferred to a amber bottle. The reagent was used freshly without storage.

3.11.5.4 Proline

Proline content in the leaves and roots of *Z. mays* and *O. sativa* was estimated according to the method of Bates et al. (1973).

Extraction: Five hundred milligram fresh tissue was homogenized in 5 mL of 3% (w/v) aqueous sulfosalicylic acid. The homogenate was transferred to the centrifuge tubes and centrifuged at 4°C for 10 min at 10,000 rpm and the supernatant was collected for the estimation of proline.

Estimation: Two mL supernatant was taken in the test tubes in triplicate and equal volume of glacial acetic acid and acid ninhydrin (2.5%) (prepared by dissolving 1.25 g of ninhydrin in a mixture of 30 mL of glacial acetic acid and 20 mL of 6 M ortho phosphoric acid) were added to it. The tubes were then heated in a water bath at 100°C for 1 h and then the tubes were placed in an ice bath. Four mL of toluene was added to the reaction mixture and stirred well using a vortex mixer. The colour intensity of chromophore-toluene layer was measured at a wavelength of 520 nm using UV-VIS Spectrophotometer (Systronics 2201, Ahmadabad, India). L-proline was used as the standard.

3.11.6 Secondary metabolites

3.11.6.1 Total phenolics

Folin-Denis reagent was used to estimate the total phenolics in the leaves and roots of *Z. mays* and *O. sativa* (Folin and Denis, 1915).

Extraction: Two hundred milligram of fresh tissue homogenized in 80% ethanol (v/v) in a clean mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 20 min and the supernatant was collected. The residue was re-extracted with 80% ethanol. The homogenate was again centrifuged and supernatant was pooled. Pooled supernatant was then dried in an oven and the residue was dissolved in 5 mL of distilled water.

Estimation: Aliquots of 500 microliter was pipetted out and made up to 2 mL with distilled water. One mL of 1 N Folin-Denis reagent was added to it. After thorough mixing, 2 mL of 20% sodium carbonate was added. This mixture was kept for colour development. The optical density of the resultant solution was measured at 700 nm. Catechol was used as the standard.

3.11.6.2 Anthocyanin content

Anthocyanin content in the leaves and roots of *Z. mays* and *O. sativa* was determined according to the method of Mancinelli et al. (1975) with some modifications.

Extraction*:* Two hundred milligram of fresh leaf and root samples was homogenized and extracted in 5 mL of acidified methanol (1:99, HCl:methanol, v/v) using a mortar and pestle. The extract was kept at 4°C for 24 h and the content was made up to 10 mL.

Estimation: Anthocyanin content was estimated from the absorbance at 530 nm using UV-VIS spectrophotometer (Systronics 2201, Ahmadabad, India) and expressed as mg g^{-1} FW.

3.11.6.3 Flavonoids

Flavonoids were extracted and measured according to the method of Mirecki and Teramura (1984).

Extraction*:* Two hundred milligram of fresh leaf samples were homogenized in a clean mortar and pestle with 5 ml of solvent containing acidified methanol (79:1:20, methanol: HCl: H_2O in v/v) and kept at room temperature for 24 h.

Estimation: The flavonoid content was determined from the absorbance of the supernatant at 315 nm using UV-VIS Spectrophotometer (Systronics 2201, Ahmadabad, India). Flavonoid content was expressed as mg g^{-1} FW.

3.11.6.4 Alkaloids

Total alkaloid content was measured by Bromocresol Green method of Talluri et al. (2018) with some modifications.

Extraction*:* Three milligram of the leaf and root samples was added into dimethyl sulphoxide (DMSO) and homogenized . The homogenate was centrifuged at 5,000 rpm for 10 min and the supernatant was collected.

Estimation: To the supernatant, 1mL of 2 N HCl was added and filtered. Then, 5 mL of bromocresol green and phosphate buffer (pH 7) was added to the filtrate. These mixtures were transferred to volumetric flasks and shaken well after adding 4 mL of chloroform. Absorbance was recorded at 470 nm. Atropine was used as the standard.

3.11.6.5 Analysis for secondary metabolites by Gas Chromatography and Mass Spectrometry (GCMS)

Sample preparation and extraction

The sample preparation and extraction method followed was according to Grover and Patni (2013) with some modifications. The leaves and roots of *Z. mays* and *O. sativa* were washed with distilled water to remove any associated debris and cut into small pieces and shade dried at room temperature for 3-4 weeks. After complete drying, the samples were grinded to fine powder with a mechanical grinder and stored in air tight container until use.

Two grams of powdered leaves and roots samples were extracted in a Soxhlet apparatus (75^{o}C for 24 h) with 20 ml of 90% aqueous methanol (v/v). After cooling, filtration was done to separate the plant residue from the extract. Further, the extract was concentrated in a rotary evaporator at 40^{0}C and stored in air tight vials at -20^{0}C until use.

GC–MS analysis

The identification of metabolites in the samples was carried out using GC-MS (Shimadzu QP2010, Kyto, Japan). An Rxi-5sil MS column (30 m length×0.25 mm ID ×0.25 μm thickness) was used for GC separation. Initially the column oven temperature was maintained at 80^{o}C for 4 min, then increased to 260^{o}C for 6 min. The instrument specifications are pressure; 65.2 kPa, injection mode; split less, total flow; 54.01 ml/min, column flow; 1.00 ml/min, solvent cut time; 6.50 min, detector gain mode; relative, injection temperature; 260^{o}C, purge flow; 3.0 mL/min and sample injection volume; 1μl. Compounds were identified in the samples by comparing with the mass spectra of National Institute of Standard and Technology (NIST 11) and WILEY 8 library.

3.12 Antioxidant defence mechanism

3.12.1 Non-enzymatic antioxidants system assay

3.12.1.1 Ascorbate (AsA) content

The estimation of AsA content was done in the leaves and roots of *Z. mays* and *O. sativa* according to the method of Chen and Wang (2002).

Extraction*:* Five hundred milligram of tissue was weighed and homogenized in 5 mL of 5% (w/v) TCA. The homogenate was centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant was for the estimation of AsA content.

Estimation*:* An aliquot of 0.3 mL of the supernatant was mixed well with 0.3

mL of 200 mM NaH_2PO_4. To this mixture, 0.5 mL of 10% (v/v) TCA, 0.4 mL of 42% (v/v) H_3PO_4, 0.4 mL of 4% (w/v) bipyridyl (dissolved in 70% alcohol) and 0.2 mL of 3% $FeCl_3$ (w/v) was added. The mixture was incubated at 42°C for 15 min. The absorbance was measured immediately after incubation at 524 nm and AsA content was calculated from a standard curve prepared using different concentrations of AsA.

3.12.1.2 Glutathione (GSH) content

The GSH content estimation was done in the leaves and roots of *Z. mays* and *O. sativa* according to the method of Chen and Wang (2002).

Extraction*:* Five hundred milligram of tissue was weighed using an electronic balance and homogenized with 6 mL 5% (w/v) TCA. The homogenate was centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant was used for the estimation of reduced glutathione content.

Estimation*:* To an aliquot of 0.5 mL of the supernatant, 2.6 mL of 150 mM NaH_2PO_4 buffer (pH 6.8) and 0.18 mL of 3 mM 5 dithio-bis-2-nitrobenzoic acid (DTNB) were added (DTNB was dissolved in 100 mM phosphate buffer, pH: 6.8). After 5 min, the absorbance was read at 412 nm and GSH content was calculated from a standard curve using varying concentrations of reduced glutathione.

3.12.2 Enzymatic antioxidant system assay

3.12.2.1 Superoxide dismutase (SOD, EC 1.15.1.1)

Estimation of SOD activity in the fresh sample was done in the leaves and roots of *Z. mays* and *O. sativa* as per the modified protocol of Giannopolitis and Ries (1977).

Extraction*:* Two hundred milligram of tissue was ground to fine powder in

a pre-chilled mortar along with liquid nitrogen and the tissue was homogenized using 50 mM phosphate buffer of pH 7.8. The homogenate was centrifuged at 14,000 rpm for 15 min at 4°C. The supernatant was used for enzyme assay.

Enzyme Assay: SOD activity was measured by monitoring the ability of SOD to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture consisted of 0.1 mL of 1.5 M sodium carbonate, 0.3 mL of 0.13 M methionine, 0.3 mL of 10 µM EDTA, 0.3 mL of 13 µM riboflavin and 0.3 mL of 0.63 mM NBT and 0.1 mL enzyme extract. The reaction mixture was made up to 3 mL using phosphate buffer (50 mM, pH 7.8). Different assay systems were set, *viz.* dark-control, light-control and test samples. Test tubes containing only assay mixture, without enzyme extract served as control. One set of control was illuminated under fluorescent lamp for 30 min (light-controls) and other one was kept in the dark (dark-control). One set of test samples (tubes containing assay mixtures with enzyme extract) was also illuminated and other set was kept in dark. The dark control served as blank and illuminated control as absolute light control. The formazan accumulation in different tubes was quantified using UV-VIS Spectrophotometer (Systronics 2201, Ahmadabad, India) by recording the absorbance of the developed blue colour at 560 nm against the blank. Results were expressed as units SOD mg^{-1} protein. One unit of SOD was defined as the enzyme activity that inhibited the photo reduction of NBT to blue formazan by 50%.

3.12.2.2 Catalase (CAT, EC 1.11.1.6)

The activity of CAT in the fresh samples was determined by following the method of Kar and Mishra (1976).

Extraction: Two hundred milligram of the plant tissue was homogenized using

a pre-chilled glass mortar and pestle in a medium consisting of 50 mM phosphate buffer (pH 7.0). The homogenate was filtered through two layered muslin cloth and was made up to 10 mL using phosphate buffer. The filtrate was then centrifuged at 16,000 rpm for 15 min at 4°C. The supernatant was used for the enzyme assay.

Enzyme assay: The activity of CAT was determined based on the decrease in absorbance at 240 nm for 1 min following the decomposition of H_2O_2. One unit of the enzyme was defined as μmol H_2O_2 decomposed per min per mg protein. Assay system consisted of 2.4 mL of 50 mM phosphate buffer (pH 7.0), 0.3 mL enzyme extract, and 0.3 mL of 30 mM hydrogen peroxide. The phosphate buffer and enzyme extract was pipetted out and mixed well in a test tube. To this, hydrogen peroxide was added to initiate the enzyme activity. Immediately after the addition of hydrogen peroxide, enzyme activity was measured at 240 nm for 90 second at 15 second interval. The CAT activity was measured in terms of μmol H_2O_2 (ε = 0.036 $mM^{-1}cm^{-1}$) oxidized per min per milligram protein.

3.12.2.3 Ascorbate peroxidase (APX, EC 1.11.1.11)

APX activity in the fresh samples was assayed by following the method of Nakano and Asada (1981).

Extraction: Five hundred milligram of fresh tissue was homogenized with 10 ml of extraction medium in a pre-chilled mortar. The extraction buffer consisted of 50 mM potassium phosphate buffer (pH 7.0), containing 0.33 M sorbitol, 1 mM $MgCl_2$, 2 mM EDTA, 10 mM NaCl, 0.5 mM KH_2PO_4 and 1 mM ascorbate.

The homogenate was filtered through two layers of cheese cloth and centrifuged at 4°C for 4 min at 14000 rpm. The pellet was discarded and the supernatant was centrifuged again at 5000 rpm for 15

min at 4°C and the supernatant the resulting supernatant was used for enzyme assay.

Enzyme Assay: Cytosolic APX activity was assayed by monitoring the decrease in absorbance at 290 nm due to AsA oxidation. The 3 ml assay system consisted of 0.5 mM AsA, 0.1 mM EDTA in 50 mM potassium phosphate buffer (pH 7.0). Twenty µL of cytosolic enzyme extract was added to the buffer and the enzyme reaction was initiated by adding 10 µL of 100 mM H_2O_2. The AsA (ε = 2.8 mM-1cm-1) content was recorded by the decrease in absorbance at 290 nm. One unit of enzyme activity was defined as the amount of enzyme that oxidized one µmol of AsA per min at room temperature.

3.12.2.4 Guaiacol peroxidase (GPOX, EC 1.11.1.7)

GPOX activity in the fresh samples was measured according to the method of Gasper et al. (1975).

Extraction: Two hundred milligram of tissue was weighed and the tissue was ground to fine powder in a pre-chilled mortar using liquid nitrogen. Ten percentage of the homogenate was prepared by grinding in 50 mM Tris-HCl buffer (pH 7.5). The extract was filtered through two layered muslin cloth. The filtrate was transferred to centrifuge tubes and centrifuged at 15,000 rpm for 15 min at 4°C. The supernatant was transferred to a test tube, stored in an ice bath, and used for enzyme assay.

Enzyme Assay: GPOX activity was measured following the H_2O_2 dependent oxidation of guaiacol (extinction coefficient 26.6 mM^{-1} cm^{-1}) at 420 nm. Three mL assay mixture consisted of 2.938 mL of 100 mM phosphate buffer (pH 7.8), 30 µL of 1% guaiacol and 20 µL of enzyme extract. Twelve microlitre of hydrogen peroxide was added to initiate the enzyme activity. Immediately after the addition of hydrogen peroxide, the increase in absorbance due to

oxidation of guaiacol was measured at 420 nm using a UV-VIS Spectrophotometer (Systronics 2201, Ahmadabad, India) for three min at intervals of 30 second. One unit of GPOX activity was defined as the amount of enzyme that caused the formation of 1 µM of tetraguaiacol per min.

$$\text{Enzyme activity (Units/mL)} = \frac{V \times (\text{Change in absorbance/min})}{\varepsilon . d . v}$$

Where, V = Total volume

ε = Extinction coefficient of substrate (that disappears) or the product (which appears). It is expressed in 1/micromoles/cm

v = Aliquot volume (mL)

d = Path distance of cuvette in cm (1 cm)

The value calculated above represent the activity of 1 mL enzyme extract in 'units' as the total number of moles of product formed or substrate disappeared in one min. Since, one enzyme unit represents one micromole of product formed or substrate disappeared in 1 min, one mL of enzyme solution would contain 'units' equivalent to that calculated from 1 mL extract. Specific activity was calculated after determining soluble protein concentration according to Bradford (1976).

$$\text{Specific activity} = \frac{\text{Activity in Units}}{\text{mg protein/mL enzyme extract}}$$

3.13 Osmolality

Osmolality of cell sap was measured according to Hura et al. (2007), using a vapor pressure osmometer (Wescor 5520, Amarillo, USA). Cell sap from tissue was collected by freeze thawing method. Two grams of sample was frozen in liquid nitrogen and then kept in a deep freezer

(-80°C) for 30 min. For the estimation, leaf and root samples were thawed at room temperature and the sap extruding from the leaf discs was collected with a 10 µL pipette and quickly transferred to the disc chamber of the osmometer and readings were recorded.

3.14 Fourier Transform Infrared (FTIR) spectroscopic analysis of lignin

Isolation of lignin

Fourier Transform Infrared (FTIR) spectroscopic analysis of lignin was performed according to the protocol of Domínguez-Robles et al. (2017). Two hundred milligram 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 60ºC 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 ton of hydraulic pressure. FTIR analysis of the samples was carried out at midinfra-red region of 400–4000 cm^{-1} (Jasco 4100, Shanghai, China). The data was interpreted using the software origin 8 (Origin Lab Corporation, One Roundhouse Plaza, Northampton, MA 01060, USA).

3.15 Quantitative estimation of Cd and Zn

Samples for quantifying Cd and Zn were prepared according to the method of Allan (1969) and were analyzed using atomic absorption spectrophotometer (Shimadzu AA-7000, Kyoto, Japan). Different plant parts *viz.* root and shoot tissues of treatments and control were sampled and dried at 60°C in a hot air oven. Known weights of the dried samples were digested by refluxing in a mixture of nitric acid and perchloric acid in the ratio of 10:4 until the solution became colourless using Kjeldahl's flask heated in a heating mantle. Then the digest was filtered and further transferred to standard flask and the volume was made up to 50 mL and stored in screw-capped containers. Heavy metals present in the digested samples were further analyzed using atomic absorption spectrophotometer. Certified standard reference materials (Merck) were used to verify the obtained results. Determination of elemental concentrations in samples was determined by extrapolation from the calibration curve. The translocation factor (TF) of Cd and Zn in *Z. mays* and *O. sativa* was determined using the equation,

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

3.16 Scanning Electron Microscopic (SEM) studies

3.16.1 Elemental distribution pattern

To study the distribution pattern of elements, an advanced, high resolution, Field Emission Scanning Electron Microscope (Gemini 300, Zeiss, Berlin, Germany) was used and the analysis were carried out in the fresh root and leaf samples of *Z. mays* and *O. sativa* exposed to 8 d of stress. In order to get a higher quality, secondary electron image for FESEM examination, dehydrated (by passing through acetone series) cross sections of the root and

leaf samples were placed onto a double-sided carbon tape. For coating it with an ultrathin electrically conducting material, gold-palladium alloy was deposited on the sample by sputter coating on Quorum sputter coater. Energy dispersive X-ray analysis (EDX) was performed with the detection–analysis-system known as INCA (Oxford Instruments INCA, Bucks, UK).

3.16.2 Anatomical parameters

For SEM studies root and leaf sections of all the plants were fixed in 2.5% glutaraldehyde, prepared in 0.1 M sodium cacodylate buffer (pH 7.2) for 5 min. Fixed specimens were rinsed twice with double distilled water and dehydrated by passing through an ascending acetone series. Five minutes incubation time was provided in each acetone series. Dried specimens were mounted on to grooves cut on aluminium stubs using double side adhesive conducting carbon tapes to expose the sections. Then the specimens were gold-palladium coated and further photomicrographs were taken using the photographic attachment of the Field Emission Scanning Electron Microscope (Zeiss Gemini 300, Berlin, Germany).

3.17 Estimation of tolerance indices and mycorrhizal dependency

The tolerance indices (TI) of AM and non-AM plants of *Z. mays* and *O. sativa* to Cd and Zn in soil (Rabie, 2005b) and mycorrhizal dependency (MD) are determined (Menge et al., 1978), respectively, as follows

$$\text{TI \%} = \left(\frac{\text{DW of plant exposed to HM stress}}{\text{DW of plants not exposed to HM stress of the respective tratments}}\right) \times 100$$

$$\text{MD \%} = \frac{(\text{DW of mycorrhizal plants} - \text{DW of non mycorrhizal plants})}{\text{DW of mycorrhizal plants of the respective treatments}} \times 100$$

Where, DW is the dry weight of plants.

3.18 Statistical analysis

Analysis of variance (ANOVA) was performed using SPSS software 16.0. Duncan's test was used to compare the means at 5% probability level. The data is an average of recordings from three independent experiments each with three replicates (*i.e.* n=9). The data represent mean ± standard error (SE). Pearson's correlation analysis was performed to evaluate the relationships between the photosynthetic variables and ROS content obtained in *Z. mays* and *O. sativa* under Cd and Zn toxicity.

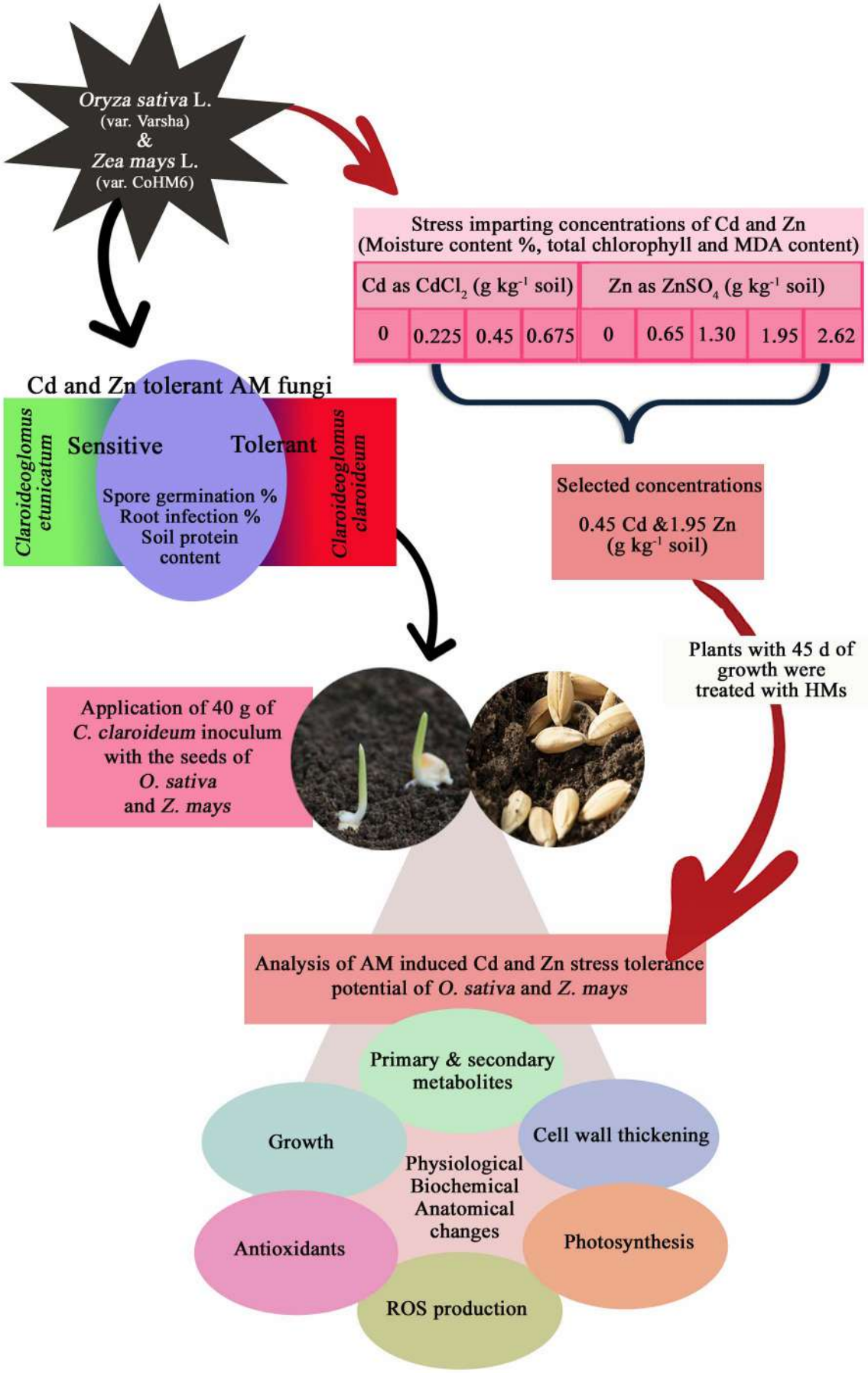

Figure 3: Schematic representation of the work

RESULTS

4.1 AM root colonization

4.1.1 Stages of AM association

The growth kinetics of *Claroideoglomus claroideum* and *C. etunicatum* was analysed in the roots of *Z. mays* and *O. sativa*. Histochemical analysis of *Z. mays* and *O. sativa* roots on 4 d of mycorrhizal association revealed that, there was 4-10% hyphal ramification (Fig. 4, 5, 6, and 7). The ramification of the hyphae increased with progress in the growth of the host plants. Hyphal ramification increased to 95-100% in the roots of *Z. mays* and *O. sativa* on 36 d of mycorrhizal association and it was maintained until 44 d of association (Table 4).

The percentage of arbuscular development drastically changed during the growth period. After a gradual increase up to 20-24 d, a dramatic reduction of arbuscular development was observed in the host plants. During initial stages (4-20 d), a gradual increase (up to 70%) in arbuscular development was observed in *Z. mays* and *O. sativa* with the association of *C. etunicatum*, but further a reduction of arbuscules to 10-25% was observed during 24-28 d. Similar trend in the arbuscular development was observed in *C. claroideum* and the arbuscular formation reduced on 24-28 d in the roots of *Z. mays* and *O. sativa*. Later in a cyclic manner, an increase in the frequency of arbuscules was observed on 44 d (Table 4).

As compared to arbuscules, vesicle formation was delayed and it started on 24-32 d (11-18%) in *O. sativa* with the association of *C. etunicatum* and *C. claroideum*, thereafter it gradually increased to 74-84% on 44 d. In *Z. mays*, the vesicular development of *C. etunicatum* and *C. claroideum* was observed during 32-36 d and it increased to 85-100% on 44 d. Similar to

vesicles, spores were also developed in the later stages of the mycorrhizal colonization. In *Z. mays*, the sporulation was started on 20 d of *C. etunicatum* association, whereas *C. claroideum* started to produce the spores on 36 d of association. The frequency of the spores was 8-10% during 20-24 d of *C. etunicatum* and *C. claroideum* association in *O. sativa*. Development of spores was increased with an increase in the period of mycorrhizal association and it increased to 40-50% in *Z. mays* on 44 d of association with both species of AM. Frequency of spores was also increased in *O. sativa* on 44 d of *C. etunicatum* (25%) and *C. claroideum* (45%) association (Table 4).

It was observed that the frequency of the hyphal ramification, vesicle formation, arbuscular development, and spore production was maximum on 44 d of mycorrhizal colonization. Therefore, 45 d of mycorrhizal association was selected for the treatment of Cd and Zn stresses.

4.1.2 Determination of optimal quantity of AM inoculum

Forty gram of AM inoculum was determined as the optimal quantity for inoculation as it was sufficient to cause 95-100% mycorrhizal association in *Z. mays* and *O. sativa* on 45 d of germination. The inoculum consisted of mycorrhizal spores, soil and root fractions of the plants. The lower quantities of AM inoculum (10, 20, and 30 g) were not sufficient to cause 100% of root colonization. On other hand, application of 50 g of AM inoculum also caused 100% mycorrhizal association, but the least quantity of inoculum with maximum efficiency was selected for further study and that was 40 g (Table 5).

4.1.3 Selection of heavy metal tolerant mycorrhizal species

For the selection of heavy metal tolerant mycorrhizal species, *Z. mays* was used as the host plant. Spore germination, root infection, and soil protein content were analyzed in *Z. mays* associated with *C. claroideum* and

C. etunicatum to determine the metal tolerant mycorrhizal species. Spore germination of *C. claroideum* and *C. etunicatum* significantly reduced due to the application of Cd and Zn stresses in the soil. The reduction in the spore germination was to the extent of 62-74% in *C. claroideum* and it was 71-80% in *C. etunicatum* on 6 d Cd and Zn stresses (Fig. 8A).

Claroideoglomus claroideum and *C. etunicatum* had maximum reduction of root infection on 2 d of spore inoculation under Cd and Zn toxicity. The reduction was 76 and 40% with *C. claroideum* exposed to Cd and Zn stresses respectively, but it was 87 and 82% with *C. etunicatum* exposed to Cd and Zn stresses respectively as compared to the control. On 6 d of spore inoculation the reduction in root infection was lesser and it was 36-45% in the case of *C. claroideum* and 47-55% in *C. etunicatum* (Fig. 8B).

In the case of soil inoculated with *C. claroideum*, 53% increase was observed in the protein content on exposure to 6 d of Cd stress, but it was decreased (38%) under Zn toxicity. At the same time, soil inoculated with *C. etunicatum* reduced the protein content on exposure to Cd and Zn stresses. The reduction was insignificant in Cd treated soil but it was to the extent of 38% in Zn treated soil (Fig. 8C).

Claroideoglomus claroideum was selected to analyse the heavy metal tolerant mechanisms of mycorrhizal plants over *C. etunicatum*, after analyzing rate of spore germination, root infection, and soil protein content.

4.2 Selection of stress imparting concentrations Cd and Zn

Zea mays and *O. sativa* were exposed to different concentrations of $CdCl_2$ (0, 0.225, 0.45, 0.675 g Cd kg^{-1} soil) and $ZnSO_4$ (0, 0.65, 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 (Fig. 9, 10, 11, and 12). Treating *Z. mays* and *O. sativa* 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 increase in the treatment period (12 d). Hence for the evaluation of mycorrhizal mediated heavy metal stress tolerance potential in *Z. mays* and *O. sativa,* the soils were supplied with 0.45 g Cd kg^{-1} and 1.95 g Zn kg^{-1} soil.

4.3 Physiological parameters

4.3.1 Fresh weight

In mycorrhizae associated *Z. mays* and *O. sativa,* a significant increase was observed in the fresh weight of both shoots and roots per plant as compared to non-AM plants in optimal conditions, but exposure of heavy metal stress reduced the fresh weight (Table 6 and 7). On exposure to Cd stress, reduction of fresh weight in non-AM and AM plants was to the extent of 73 and 40% respectively in *Z. mays* as compared to the control plants. Zinc caused 56% reduction in the fresh weight of non-AM plants, but Zn did not significantly reduce the fresh weight of AM plants (Table 6). The shoot fresh weight of *O. sativa* significantly decreased to the extent of 31% in AM plants and 45% in non-AM plants on exposure to 12 d of Cd stress. At the same time, fresh weight of non-AM and AM plants was maintained without any significant decrease under Zn stress (Table 7).

In the case of *Z. mays*, root fresh weight per plant was increased and the increase was negligible in non-AM plants but increased significantly (211-

212%) in AM plants under Cd and Zn stresses (Table 6). The root fresh weight of *O. sativa* reduced under Cd stress and the reduction was 45 and 31% in non-AM and AM plants respectively. But, the reduction was negligible in non-AM and AM plants exposed to 12 d of Zn stress (Table 7).

4.3.2 Dry weight

Mycorrhizal association increased the shoot dry weight per plant of *Z. mays* and *O. sativa* in optimal conditions, but exposure to heavy metal stresses reduced the shoot dry weight (Table 6 and 7). In the shoot of non-AM plants of *Z. mays*, Cd and Zn stresses induced reduction in the dry weight was insignificant on 12 d of treatment. However, the dry weight of the mycorrhizal plants increased significantly (86-145%) under the metal exposed condition as compared to the control (Table 6). The shoot dry weight of *O. sativa* was decreased under Cd stress; the reduction was 22 and 16% in non-AM and AM plants respectively. Under Zn stress, the reduction was 10% in non-AM plants, contradictory to this, in AM plants the shoot dry weight was increased (37%) as compared to the control (Table 7).

The dry weight of *Z. mays* roots reduced to the extent of 49-50% in non-AM plants exposed to Cd and Zn stresses. But the root dry weight increased 8-9 fold in the mycorrhizal plants exposed to Cd and Zn stresses (Table 6). The root dry weight of *O. sativa* was reduced in non-AM plants to the extent of 54 and 27% under Cd and Zn stresses respectively, but in AM plants 127 and 45% increase was recorded under Cd and Zn stresses respectively (Table 7).

4.3.3 Tissue moisture content

Heavy metal stress significantly reduced the moisture content of the shoot system (Table 6 and 7). The shoot tissue moisture content of *Z. mays* reduced (32-37%) in non-AM plants under Cd and Zn stresses, but the

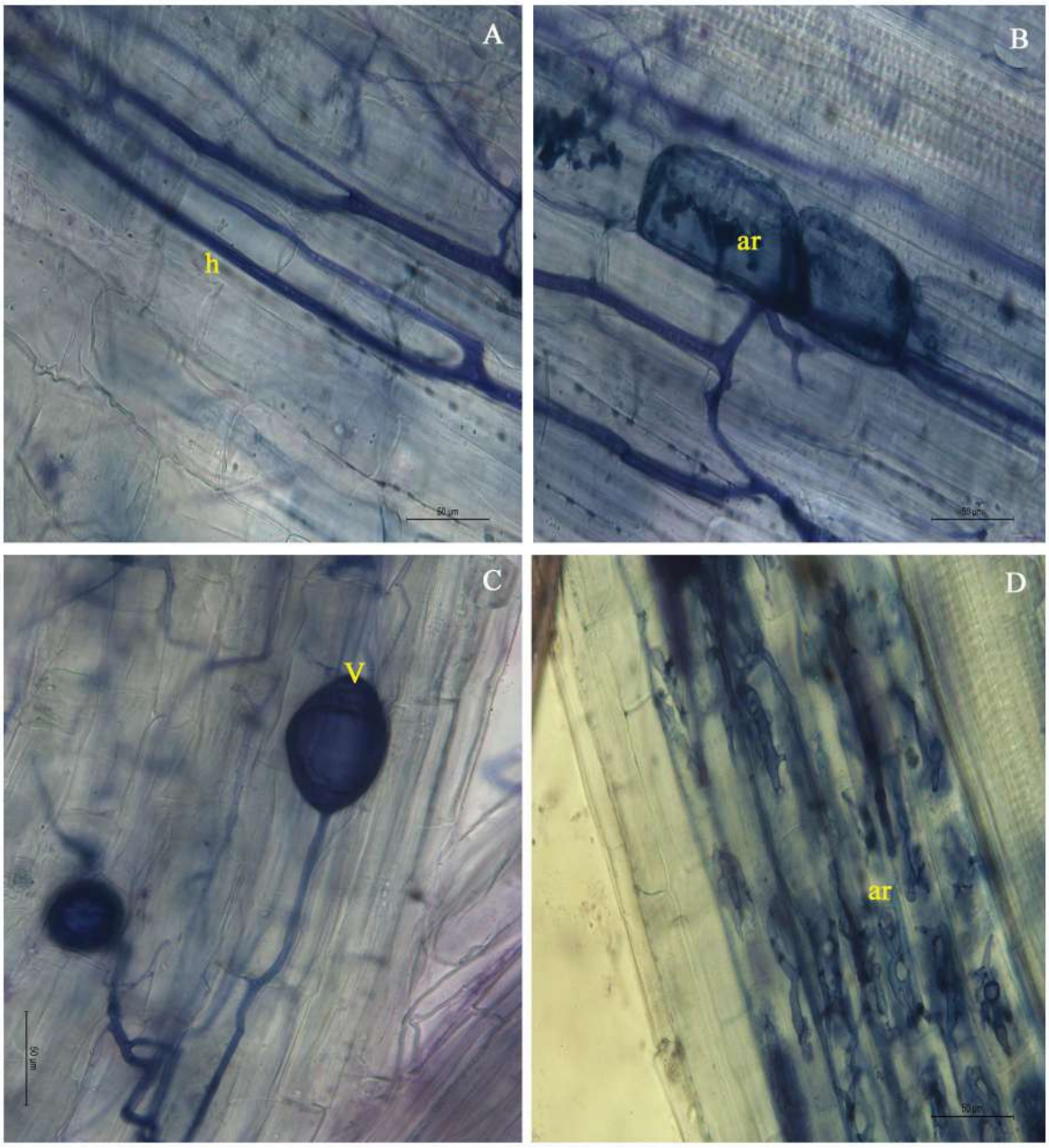

Figure 4: Photomicrographs of successive colonization of *C. claroideum* in *Z. mays* roots; h-hyphae, ar-arbuscules, and v-vesicles.

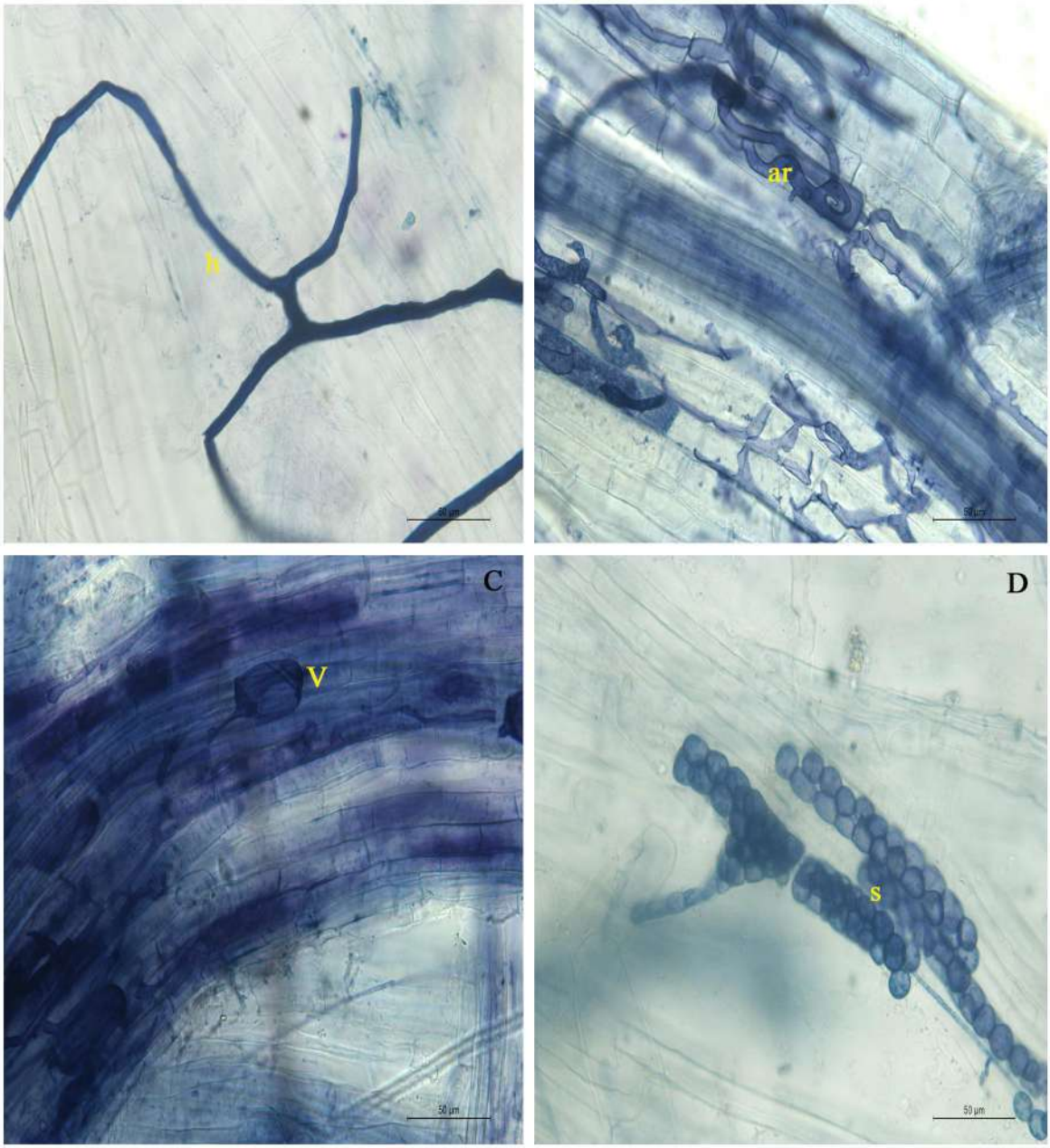

Figure 5: Photomicrographs of successive colonization of *C. claroideum* in *O. sativa* roots; h-hyphae, ar-arbuscules, s-spores and v-vesicles.

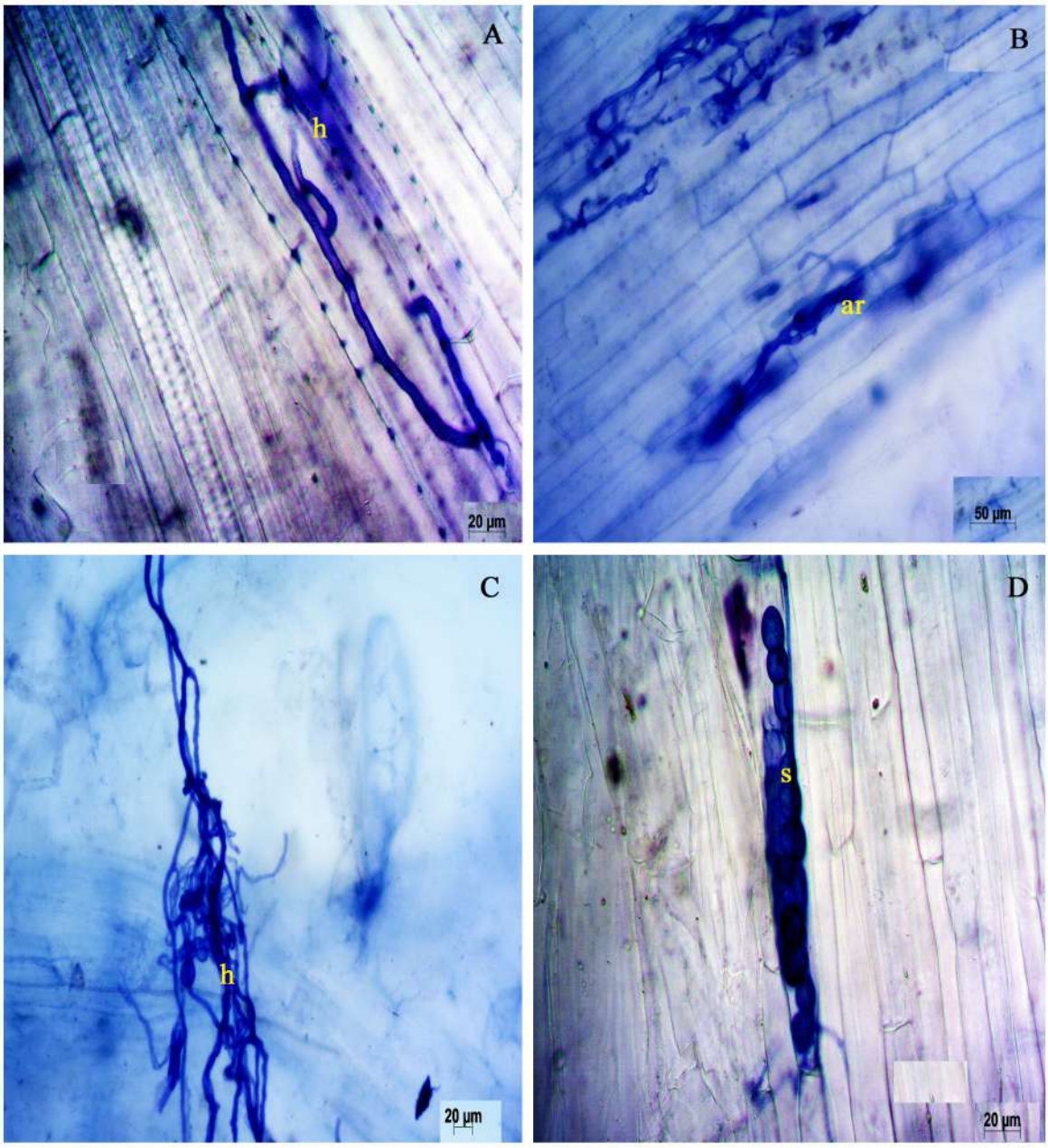

Figure 6: Photomicrographs of successive colonization of *C. etunicatum* in *Z. mays* roots; h-hyphae, ar-arbuscules, s-spores and v-vesicles.

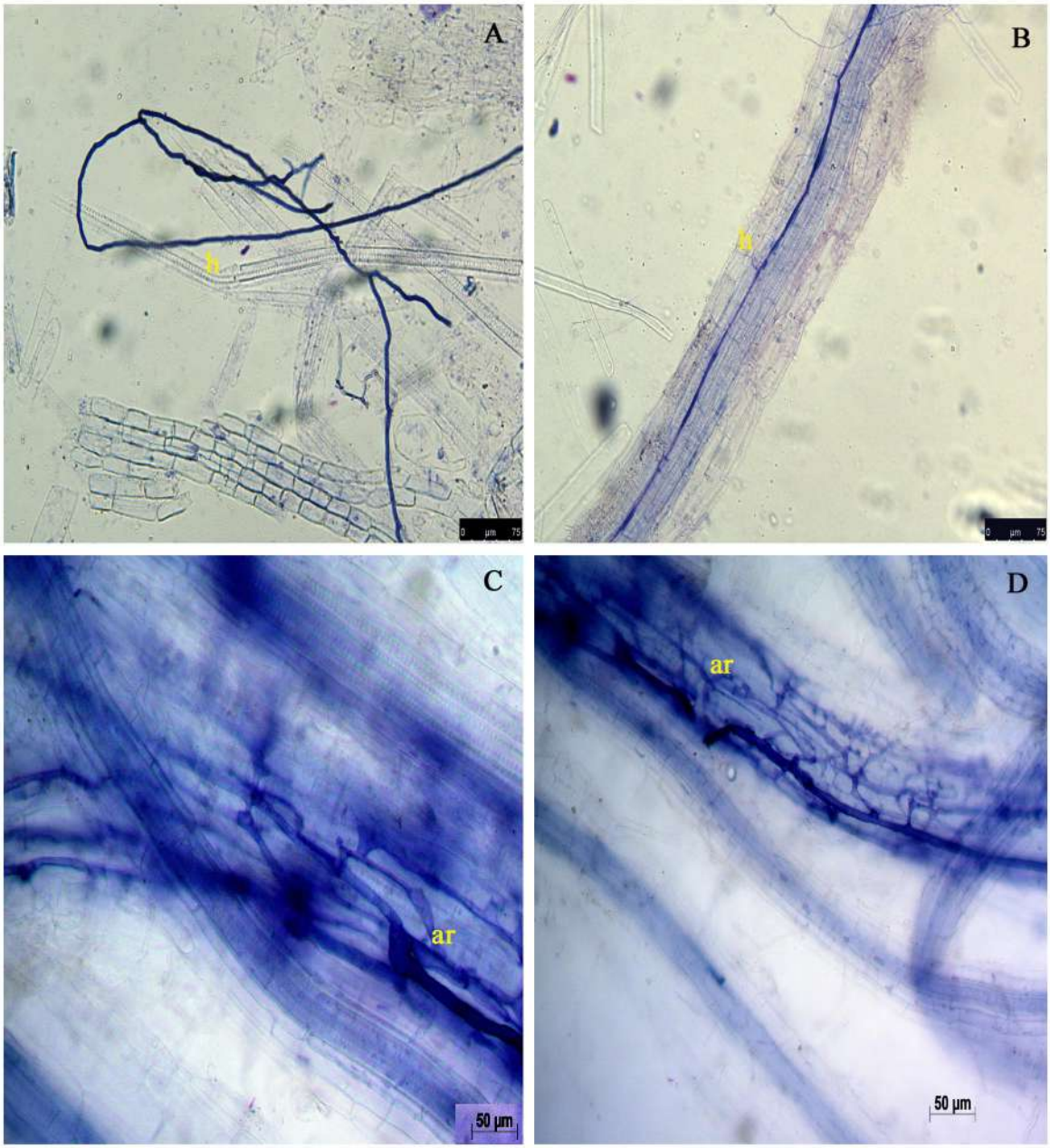

Figure 7: Photomicrographs of successive colonization of *C. etunicatum* in *O. sativa* roots; h-hyphae, ar-arbuscules, and v-vesicles.

Table 4: Growth kinetics of *C. etunicatum* and *C. claroideum* in association with the roots of *Z. mays* and *O. sativa.* Values are expressed as mean ± SE of three independent experiments. Different alphabetical letters indicates significant difference between treatments (Duncan's test, $p \leq 0.05$).

Days	Plants	Growth kinetics of *C. etunicatum*					Growth kinetics of *C. claroideum*				
		Hyphae (%)	Arbuscules (%)	Vesicle (%)	Spores (%)	Root colonization (%)	Hyphae (%)	Arbuscules (%)	Vesicle (%)	Spores (%)	Root colonization (%)
4	*Z. mays*	8 ± 2^{f}	-	-	-	8 ± 1^{f}	10 ± 1^{e}	10 ± 1^{f}	-	-	10 ± 1^{f}
	O. sativa	4 ± 1^{h}	-	-	-	4 ± 1^{f}	6 ± 1^{g}	-	-	-	6 ± 1^{g}
8	*Z. mays*	12 ± 2^{e}	8 ± 1^{g}	-	-	12 ± 2^{e}	20 ± 3^{d}	18 ± 2^{f}	-	-	20 ± 1^{e}
	O. sativa	8 ± 1^{g}	-	-	-	8 ± 1^{e}	12 ± 2^{f}	-	-	-	12 ± 2^{f}
12	*Z. mays*	50 ± 4^{f}	15 ± 1^{f}	-	-	50 ± 4^{f}	28 ± 4^{d}	12 ± 1^{f}	-	-	28 ± 1^{d}
	O. sativa	25 ± 2^{d}	10 ± 2^{e}	-	-	25 ± 2^{d}	25 ± 1^{e}	10 ± 1	-	-	25 ± 2e
16	*Z. mays*	70 ± 3^{e}	40 ± 4^{e}	-	-	70 ± 3^{e}	50 ± 5^{c}	36 ± 3^{d}	-	-	50 ± 5^{c}
	O. sativa	41 ± 2^{c}	50 ± 6^{b}	-	-	41 ± 2^{c}	38 ± 4^{d}	7 ± 1^{f}	-	-	38 ± 2^{d}
20	*Z. mays*	80 ± 8^{b}	70 ± 5^{b}	-	10 ± 1^{c}	80 ± 5^{b}	82 ± 6^{b}	15 ± 3^{f}	-	-	82 ± 2^{b}
	O. sativa	78 ± 6^{d}	70 ± 3^{a}	-	-	78 ± 2^{b}	51 ± 4^{c}	12 ± 2^{e}	-	10 ± 1^{d}	51 ± 4^{c}
24	*Z. mays*	85 ± 5^{b}	52 ± 4^{d}	-	10 ± 1^{c}	85 ± 7^{b}	89 ± 4^{ab}	59 ± 7^{b}	-	-	89 ± 3^{ab}
	O. sativa	82 ± 8^{d}	10 ± 1^{e}	11 ± 1^{d}	8 ± 2^{c}	82 ± 4^{b}	86 ± 5^{b}	84 ± 11^{a}	-	18 ± 1^{c}	86 ± 4^{b}
28	*Z. mays*	81 ± 2^{b}	25 ± 1^{f}	-	18 ± 1^{b}	81 ± 6^{b}	92 ± 6^{a}	29 ± 4^{e}	11 ± 1^{d}	-	92 ± 5^{a}
	O. sativa	88 ± 9^{c}	40 ± 1^{c}	42 ± 3^{c}	13 ± 2^{b}	88 ± 5^{b}	88 ± 3^{ab}	12 ± 1^{e}	-	20 ± 1^{b}	88 ± 3^{b}
32	*Z. mays*	99 ± 7^{b}	19 ± 1^{f}	20 ± 4^{b}	18 ± 3^{b}	99 ± 7^{b}	90 ± 7^{a}	18 ± 1^{f}	15 ± 1^{d}	-	90 ± 8^{a}
	O. sativa	94 ± 8^{b}	54 ± 7^{b}	40 ± 2^{c}	12 ± 3^{b}	94 ± 11^{a}	87 ± 7^{b}	59 ± 2^{c}	15 ± 1^{d}	38 ± 3^{a}	87 ± 4^{b}
36	*Z. mays*	100 ± 2^{a}	76 ± 5^{b}	30 ± 1^{c}	52 ± 4^{a}	100 ± 5^{a}	85 ± 7^{b}	80 ± 4^{a}	22 ± 1^{c}	11 ± 1^{c}	85 ± 8^{b}
	O. sativa	95 ± 2^{b}	29 ± 1^{d}	41 ± 3^{c}	10 ± 1^{b}	95 ± 4^{a}	92 ± 5^{a}	59 ± 3^{c}	29 ± 1^{c}	25 ± 1^{b}	92 ± 1^{a}
40	*Z. mays*	100 ± 3^{a}	35 ± 5^{e}	70 ± 1^{c}	50 ± 4^{a}	100 ± 6^{a}	98 ± 6^{a}	40 ± 6^{c}	44 ± 1^{b}	39 ± 4^{a}	98 ± 5^{a}
	O. sativa	98 ± 3^{a}	58 ± 3^{b}	49 ± 5^{b}	14 ± 5^{b}	93 ± 3^{a}	100 ± 6^{a}	38 ± 1^{d}	59 ± 5^{b}	38 ± 2^{a}	98 ± 3^{a}
44	*Z. mays*	100 ± 1^{a}	100 ± 2^{a}	100 ± 3^{a}	50 ± 3^{a}	100 ± 2^{a}	100 ± 6^{a}	51 ± 1^{b}	85 ± 4^{a}	41 ± 1^{a}	100 ± 4^{a}
	O. sativa	100 ± 1^{a}	62 ± 3^{ab}	74 ± 8^{a}	25 ± 2^{a}	100 ± 3^{a}	100 ± 12^{a}	74 ± 8^{b}	84 ± 4^{a}	45 ± 5^{a}	100 ± 4^{a}

Table 5: The rate of mycorrhizal colonization in *Z. mays* and *O. sativa* subjected to different quantites of *C. etunicatum* and *C. claroideum* inoculum. Values are expressed as mean ± SE of three independent experiments. Different alphabetical letters indicates significant difference between treatments (Duncan's test, $p \leq 0.05$).

Plant	Mycorrhiza	Mycorrhizal structures (%)	Quantity of mycorrhizal inoculum (g)				
			10	20	30	40	50
Z. mays	*C. etunicatum*	Infection	22 ± 1.1^{d}	60 ± 3^{c}	81 ± 4.05^{b}	95 ± 4.75^{a}	100 ± 5^{a}
		Hyphae	22 ± 0.66^{d}	60 ± 1.8^{c}	80 ± 2.4^{b}	95 ± 4.75^{a}	100 ± 5^{a}
		Arbuscules	20 ± 1^{d}	50 ± 2.5^{b}	60 ± 3^{a}	40 ± 2^{c}	26 ± 1.3^{d}
		Vesicles	-	20 ± 1b	40 ± 2^{a}	11 ± 0.55^{c}	-
		Spores	-	10 ± 0.5^{d}	40 ± 2^{c}	60 ± 3^{b}	70 ± 3.5^{a}
	C. claroideum	Infection	30 ± 1.5^{c}	60 ± 3^{b}	60 ± 3^{b}	95 ± 4.75^{a}	100 ± 5^{a}
		Hyphae	30 ± 1.5^{c}	60 ± 3^{b}	60 ± 3^{b}	99 ± 4.95^{a}	100 ± 5^{a}
		Arbuscules	30 ± 1.5^{c}	10 ± 1^{e}	60 ± 3^{b}	85 ± 4.25^{a}	12 ± 0.6^{d}
		Vesicles	-	-	30 ± 1.5^{b}	50 ± 2.5^{a}	12 ± 0.6^{c}
		Spores	10 ± 0.5^{c}	40 ± 2^{b}	70 ± 3.5^{a}	10 ± 0.5^{c}	40 ± 2^{b}
O. sativa	*C. etunicatum*	Infection	10 ± 0.5^{d}	40 ± 2^{c}	70 ± 3.5^{b}	93 ± 4.65^{a}	90 ± 4.5^{a}
		Hyphae	10 ± 0.5^{d}	30 ± 1.5^{c}	70 ± 3.5^{b}	90 ± 4.5^{a}	90 ± 4.5^{a}
		Arbuscules	-	-	40 ± 2^{a}	-	-
		Vesicles	-	-	-	-	10 ± 0.5^{a}
		Spore	-	-	10 ± 2^{b}	13 ± 1^{ab}	15 ± 0.5^{a}
	C. claroideum	Infection	2.5 ± 0.12^{c}	20 ± 1^{b}	100 ± 5^{a}	90 ± 4.5^{a}	95 ± 4.75^{a}
		Hyphae	2 ± 0.1^{d}	20 ± 1^{c}	95 ± 4.75^{a}	90 ± 4.5^{b}	98 ± 4.9^{a}
		Arbuscules	-	-	-	25 ± 1.2 ^{a}	-
		Vesicle	-	-	33 ± 1.65^{a}	10 ± 0.5^{b}	10 ± 0.5^{b}
		Spores	-	-	70 ± 3.5^{a}	40 ± 2^{b}	-

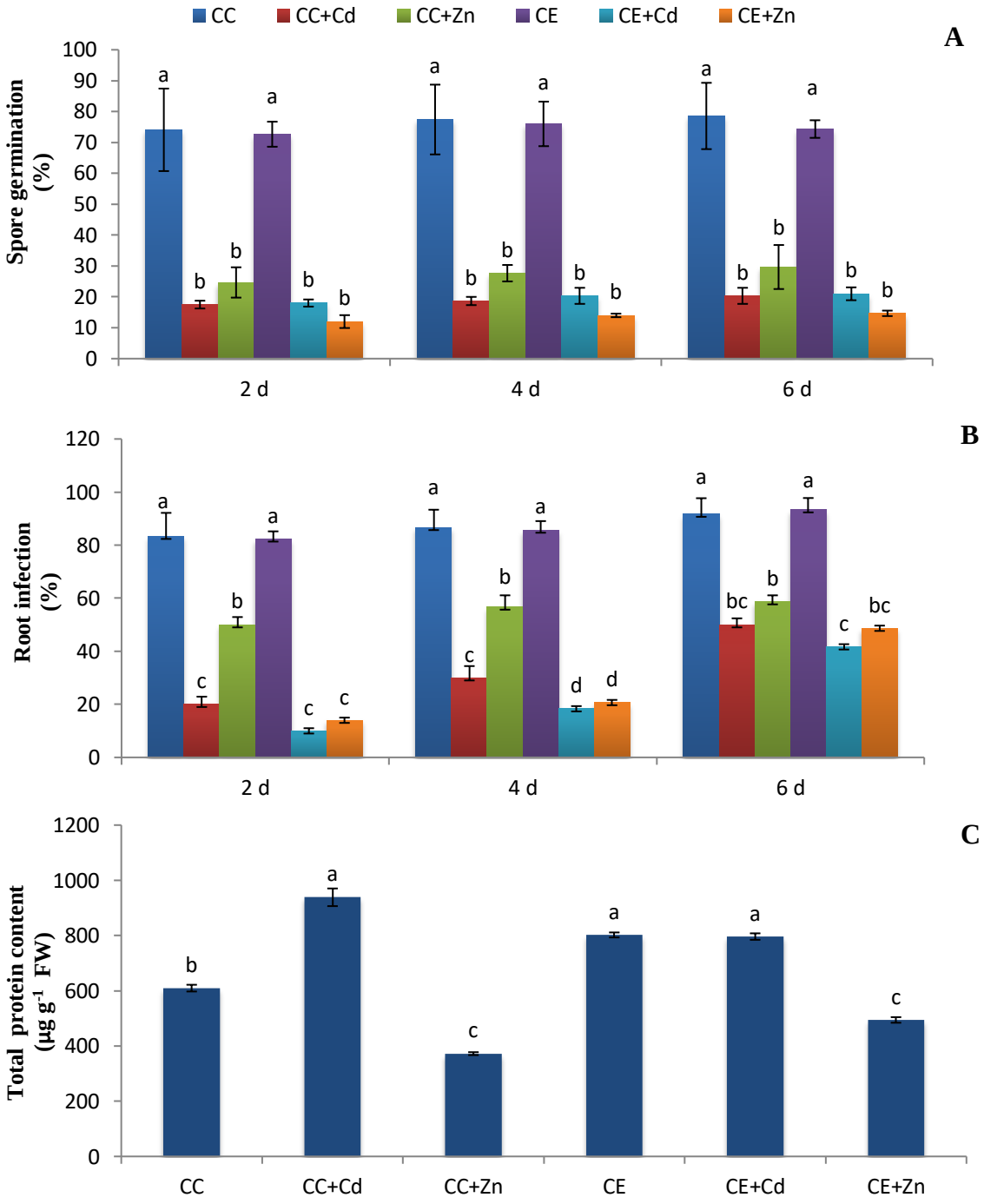

Figure 8: A) spore germination %, B) root infection %, and C) total protein content in rhizospheric soil of *Z. mays* associated with *C. claroideum* (CC) and *C. etunicatum* (CE) exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

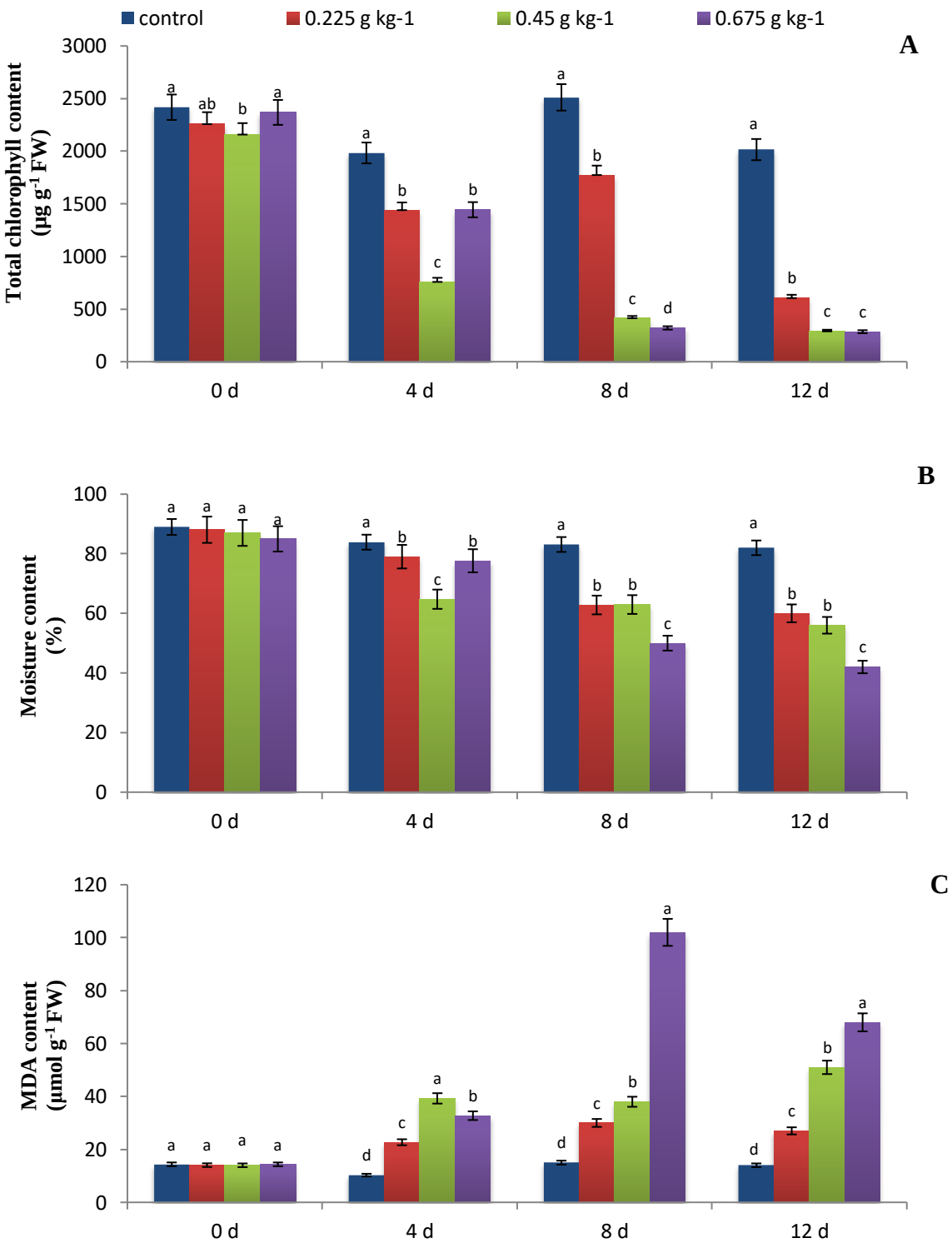

Figure 9: A) total chlorophyll content, B) moisture content % and C) MDA content in the leaves of *Z. mays* treated with Cd (0.225, 0.45, 0.675 g Cd kg^{-1} soil). Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

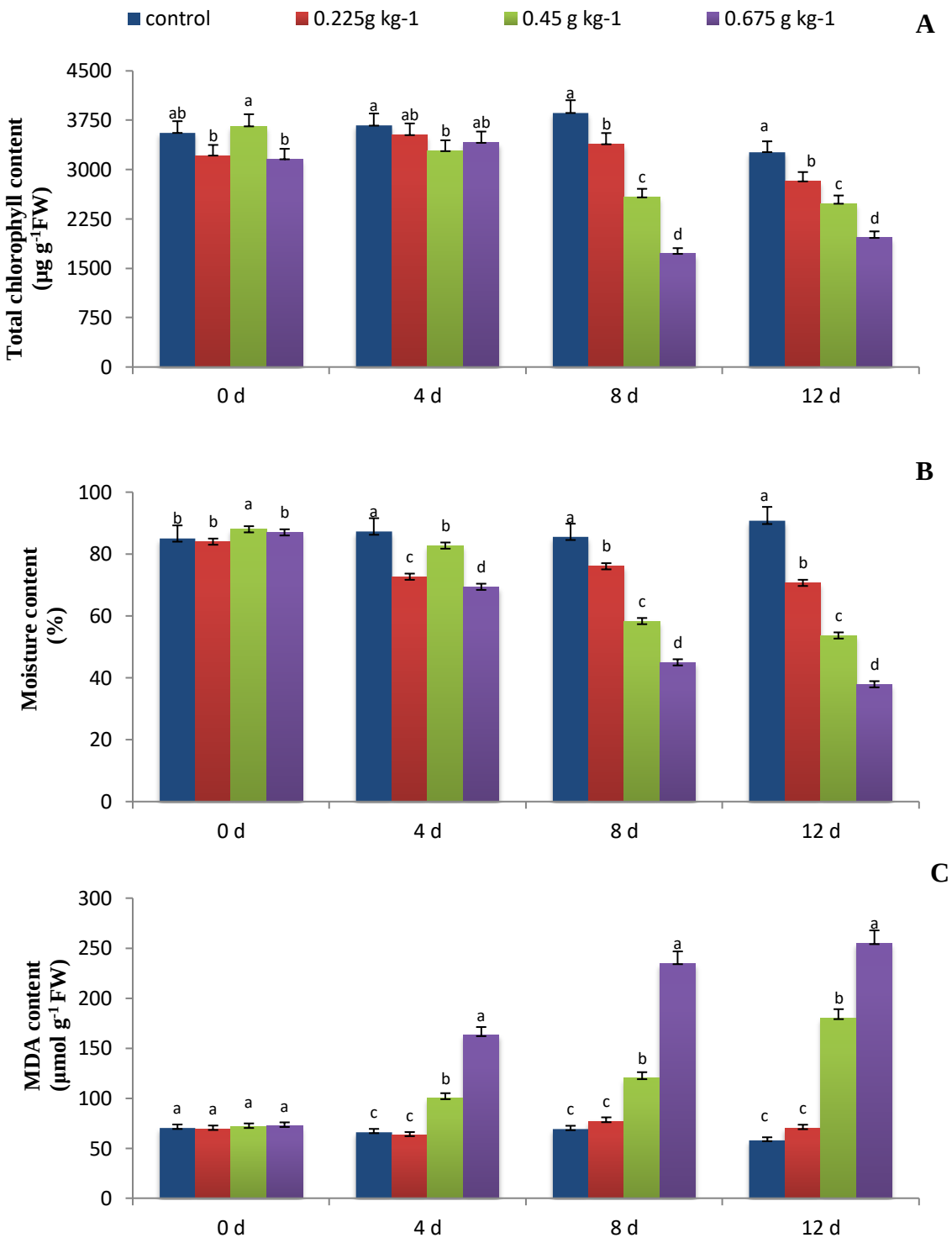

Figure 10: A) total chlorophyll content, B) moisture content and C) MDA content in the leaves of *O. sativa* treated with Cd (0.225, 0.45, 0.675 g Cd kg^{-1}soil). Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

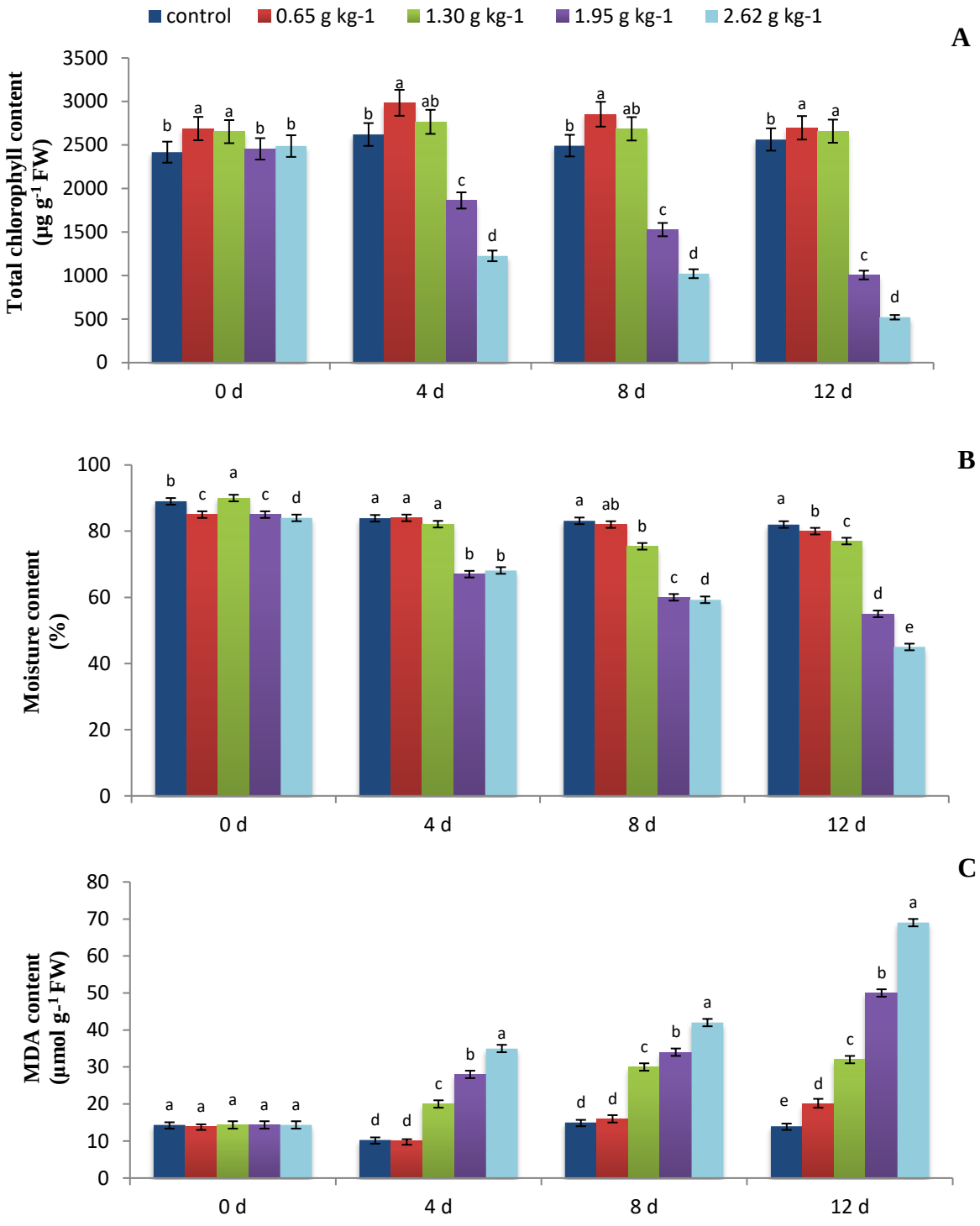

Figure 11: A) total chlorophyll content, B) moisture content and C) MDA content in the leaves of *Z. mays* treated with Zn (0.65, 1.30, 1.95, 2.62 g Zn kg^{-1}soil). Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

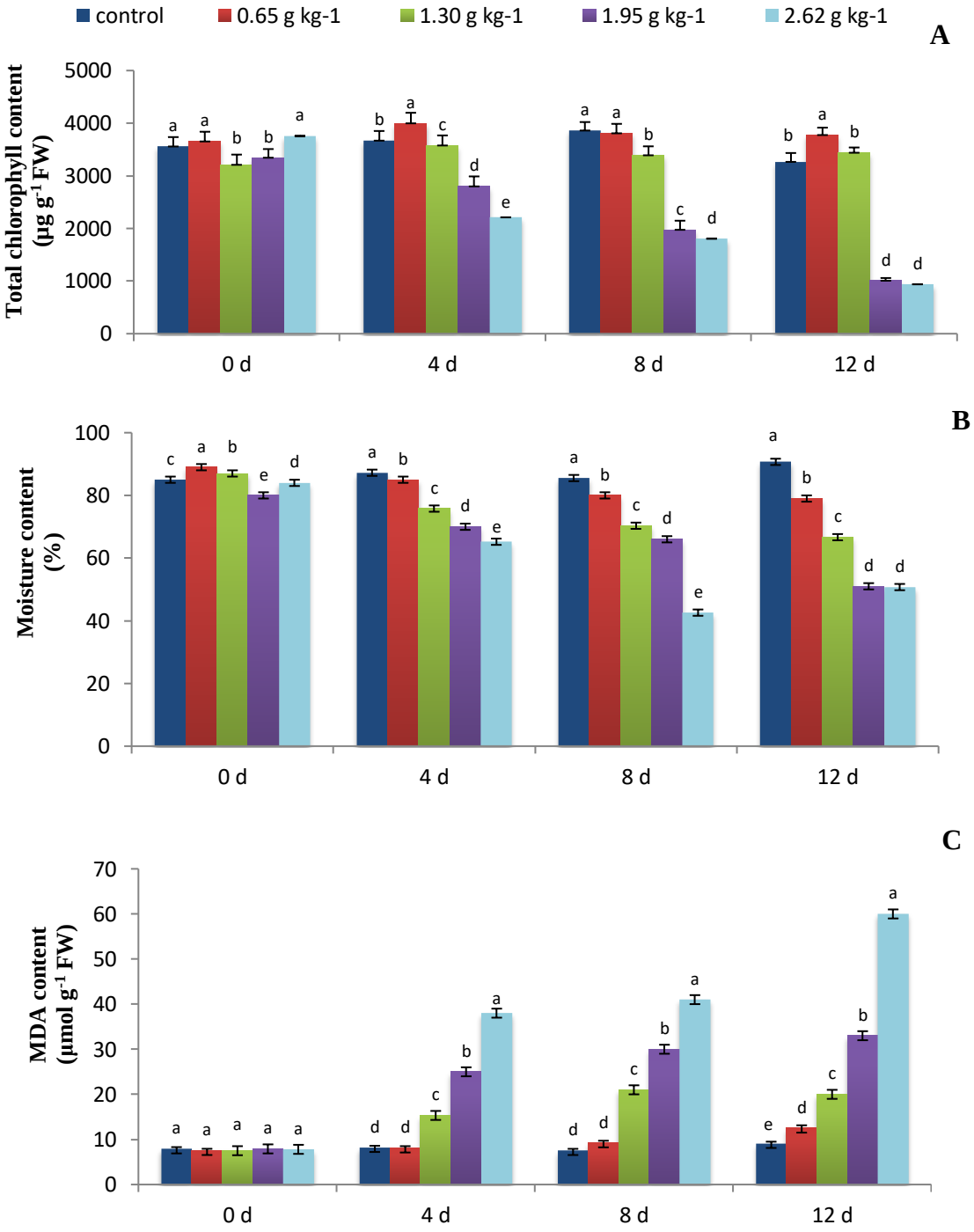

Figure 12: A) total chlorophyll content, B) moisture content % and C) MDA content in the leaves of *O. sativa* treated with Zn (0.65, 1.30, 1.95, 2.62 g Zn kg^{-1} soil). Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

reduction was only to the extent of 8-25% in AM plants (Table 6). The shoot moisture content of *O. sativa* reduced slightly in non-AM and AM plants exposed to Cd and Zn stresses on 12 d (Table 7).

On exposure to Cd and Zn stresses, the root moisture content of *Z. mays* was slightly increased in non-AM plants, but it was decreased in AM plants (Table 6). On exposure to Cd and Zn stresses, the moisture content of root tissue was increased, and the increase was higher in non-AM plants as compared to AM *O. sativa* (Table 7).

4.3.4 Root volume and viability

Root volume of *Z. mays* and *O. sativa* was significantly increased in AM plants as compared to non-AM plants. Root volume of *Z. mays* without AM association reduced by 27% on exposure to Cd and Zn stresses, but in the case of AM plants, the root volume increased to the extent of 48 and 84% on 12 d of Cd and Zn stresses respectively. Root volume significantly decreased in *O. sativa* plants under Cd and Zn stresses and the decrease was to the extent of 54 and 31% respectively in non-AM plants as compared to control. At the same time, root volume of AM plants was maintained equal to that of control even on 12 d of Cd and Zn stresses (Table 8).

Heavy metal stress increased the uptake of Evans blue stain in the roots of *Z. mays* and *O. sativa.* In the roots of *Z. mays,* the increase was 203 and 162% in non-AM and AM plants respectively on 12 d of Cd stress. Zinc toxicity also increased the Evans blue stain uptake to the extent of 122 and 51% in non-AM and AM plants respectively. The most significant amount of Evans blue uptake was detected in the roots of non-AM *O. sativa* plants exposed to Cd and Zn stresses and it was 141 and 216% respectively. But it was only 33 and 171% in the roots of AM associated plants under Cd and Zn stresses (Fig. 13).

4.3.5 Photosynthesis

Cadmium and Zn stresses induced reduction in chlorophyll content, carotenoid content and photosystems (I and II) activities, and the reduction was higher in non-AM plants as compared to AM plants.

4.3.5.1 Chlorophylls and carotenoids content

In *Z. mays* and *O. sativa* plants subjected to Cd and Zn stresses, a reduction in chlorophyll as well as carotenoids contents was observed (Fig. 14). The reduction in total chlorophyll and carotenoids contents were increased with an increase in the days of heavy metal exposure. Sixty percentage of reduction in total chlorophyll content was observed in non-AM and AM plants of *Z. mays* subjected to Cd stress (12 d). At the same time, the reduction induced by Zn was to the extent of 70-73% in non-AM and AM plants. Similarly, the reduction of carotenoid content in non-AM and AM plants of *Z. mays* was 59-65% under 12 d of Cd and Zn stresses (Fig. 14A and C).

On 4 d of stress, the reduction in the total chlorophyll content of *O. sativa* was prominent in non-AM plants (24-27%), but AM plants did not exhibit any significant reduction as compared to the control. The reduction of total chlorophyll content of *O. sativa* was increased to 52-60% in non-AM and AM plants on 12 d of imparting Cd stress, and under Zn stress, the reduction was 49 and 29% in non-AM plants and AM plants respectively. At the same time, 33-38% of reduction was observed in the carotenoid content of non-AM and AM *O. sativa* plants on exposure to 12 d of Cd and Zn toxicity (Fig. 14B and D).

4.3.5.2 Chlorophyll *a* fluorescence induction curve and JIP parameters

The modifications in the light-dependent photosynthetic process of *Z. mays* and *O. sativa* under Cd and Zn stresses were observed in the shape of induction curves of chlorophyll fluorescence (Fig. 15). Both metals did not significantly affect the standard shape of OJIP (chlorophyll *a* fluorescence transient) curves in *O. sativa* plants (Fig. 15B). However, Cd and Zn toxicity significantly modified the OJIP curve of *Z. mays* as compared to the control plants (Fig. 15A). At the same time, mycorrhization aid to maintain the standard shape of OJIP curves in *Z. mays* and *O. sativa* under Cd and Zn stresses. In *O. sativa*, both Cd and Zn stresses did not cause any major changes in initial fluorescence (F_O). Whereas in *Z. mays*, the F_O drastically reduced in the non-AM plants exposed to Cd stress, but not in Zn treated and mycorrhizae associated ones. In the case of maximum fluorescence (F_M), a reduction was observed in non-AM plants of *Z. mays* treated with Cd and Zn stresses, and the reduction was to the extent of 80 and 18% respectively over the control plants. But, the reduction was only 37 and 3% in AM plants exposed to Cd and Zn toxicity respectively. In non-AM *O. sativa* plants, the Cd and Zn stresses induced a reduction in F_M to an extent of 24-32%, but the reduction was insignificant in AM plants subjected to Cd and Zn stresses (Fig. 15B). Different from that of Zn treated AM plants, the F_O-F_J (F_J-chlorophyll fluorescence transient at 2 ms) phase of the transient curve reduced in the Zn treated non-AM plants and Cd treated non-AM as well as AM plants of *Z. mays*. In *O. sativa* plants, the F_O-F_J phase reduced in non-AM plants exposed to Cd and Zn stresses. In *Z. mays*, F_I-F_P (F_I-chlorophyll fluorescence transient at 30 ms) phase was also reduced in Cd and Zn treated non-AM plants and Cd treated AM plants (Fig. 15A). In *O. sativa*, the non-AM plants exposed to Cd and Zn stresses significantly reduced the F_I-F_P phase (Fig. 15B).

Pronounced changes during Cd and Zn stresses were also observed in the radar plot representing yield parameters and performance index (Fig. 16). However, the most obvious negative influence of heavy metal stress was observed in the case of non-AM *Z. mays* and *O. sativa* plants exposed to Cd and Zn stresses, wherein the reduction in area above the induction curve was prominent (Fig. 16A and B).

Maximum efficiency of water splitting complex (F_V/F_O) of non-AM and AM *Z. mays* plants was also decreased under Cd and Zn stresses in the range of 59-67% and 32-33% in Cd and Zn treated plants respectively. F_V/F_O was decreased in *O. sativa* plants exposed to Cd and Zn stresses, and a prominent reduction was observed in non-AM plants exposed to Cd stress (23%). The changes in tF_M (time taken to reach F_M) was another metal induced modification observed in *O. sativa* and *Z. mays* plants. In *Z. mays* plants, tF_M was increased in Cd treated non-AM plants and Zn treated AM plants, and no specific modification was observed in non-AM plants exposed to Cd toxicity. At the same time, the tF_M was decreased by 19% in AM plants exposed to Cd stress. tF_M was increased to 22-24% in non-AM and AM *O. sativa* plants exposed to Cd stress, and the increase was 13-26% in *O. sativa* plants exposed to Zn stress (Fig. 16A and B).

Metal induced modification in N (turn over number of Q_A) was visualized in the radar plot of *O. sativa* and *Z. mays* plants. In *O. sativa* plants, the reduction of N was up to 21-25% in non-AM plants exposed to Cd and Zn stresses. But it was only 6-17% in AM plants. In the case or *Z. mays*, the Cd induced reduction in N was 42 and 7% in non-AM and AM plants respectively. At the same time, under Zn stress, 8 and 17% of reduction was observed in non-AM and AM plants respectively as compared to the control plants (Fig. 16A and B).

SFI_{ABS} and 10RC/ABS were also reduced in Cd and Zn treated *Z. mays* as well as *O. sativa* plants. In Cd treated non-AM and AM *Z. mays* plants and non-AM *O. sativa* plants prominent reduction in SFI_{ABS} and 10RC/ABS was recorded. DF_{TOTAL} (PSII-relative driving force) was decreased in Cd treated non-AM plants and Zn treated non-AM and AM *Z. mays* plants, but it was increased in Cd treated AM plants. In *O. sativa* plants, the DF_{TOTAL} decreased drastically in non-AM plants as compared to AM plants under Cd and Zn stresses (Table 9).

The Fv/F_M values measured in the leaves of Cd and Zn treated non-AM and AM *Z. mays* and *O. sativa* plants reduced from that of the value recorded in the control. The reduction in Fv/F_M was 24-30% in non-AM and AM *Z. mays* plants on exposure to Cd stress, and it was only 9% under Zn stress. In the case of *O. sativa* plants, the reduction in Fv/F_M was insignificant in both non-AM and AM plants subjected to Cd and Zn stresses (Table 9).

In *Z. mays* plants, the reduction in PI_{ABS} was 89-96% in non-AM and AM plants exposed to Cd, but it was only 64-65% in Zn treated non-AM and AM plants. PI_{ABS} in the non-AM plants of *O. sativa* reduced by 65 and 54% on exposure to Cd and Zn stresses respectively but the reduction was only 28 and 11% in AM plants exposed to Cd and Zn stresses respectively (Fig. 16A and B). In the case of PI_{TOTAL}, the reduction was 67 and 48% in Cd treated non-AM and AM *Z. mays* plants respectively. Under Zn stress, 29-36% of reduction in PI_{TOTAL} was observed in non-AM and AM plants. When *O. sativa* plants were exposed to Cd stress, the reduction of PI_{TOTAL} was 72 and 50% in non-AM and AM plants respectively. On exposure to Zn stress, 69 and 38% of reduction was observed in non-AM and AM plants respectively (Table 9).

Yield parameters also significantly varied under Cd and Zn stresses and prominent variations were observed in φ(Po), φ(Eo), and ψo. Of these,

φPo was slightly reduced in non-AM plants of *O. sativa* but AM plants did not exhibit any reduction as compared to control, under Cd and Zn toxicity. In non-AM and AM *Z. mays* plants, Cd stress caused 23-30% reduction of φPo, but Zn stress caused only 9% of reduction as compared to the control. The reduction in φ(Eo) was 48-30% in non-AM plants of *O. sativa* exposed to Cd and Zn stresses, but it was only 4-14% in AM plants. In *Z. mays* plants, the reduction in φ(Eo) was 69-83% in non-AM and AM plants exposed to Cd stress. When exposed to Zn stress, the reduction was only to the extent of 34-40%. In *O. sativa*, the reduction of ψo was 46 and 28% in non-AM plants exposed to Cd and Zn stresses respectively, but the reduction was insignificant in AM plants. In the case of *Z. mays* plants, 59-74% of reduction in ψo was observed under Cd stress and 27-34% reduction was observed under Zn stress in non-AM and AM plants. Reduction in the yield parameters were predominantly observed in the Cd treated *Z. mays* plants as compared to *O. sativa* plants (Fig. 16A and B).

Phenomenological energy pipeline models help to visualize the intensity of Cd and Zn induced modifications in overall photochemistry of non-AM and AM plants, and it also interrupt the stepwise flow of energy through PSII at the level of a cross-section (CS) (ABS/CS_M, TRo/CS_M, ETo/CS_M, and DIo/CS_M) as well as at reaction center (RC) level (ABS/RC, TR/RC, ET/RC, and DI/RC) (Fig. 17, 18, 19, and 20). CS indicates the surface of the excited portion of the photosynthetic sample, whereas RC represents photochemically active reaction centers of PSII that can reduce Q_A, so the former includes the photosynthetic response of both active and inactive reaction centers. The phenomenological energy flux parameters such as ABS/CS_M, TRo/CS_M, and ETo/CS_M were lower in the plants exposed to Cd and Zn stresses (Fig. 17 and 18).

Table 6: Fresh weight, dry weight, and moisture content % in shoot and root of non-AM and AM *Z. mays* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Values are expressed as mean ± SE of three independent experiments. Different alphabetical letters indicates significant difference between treatments (Duncan's test, $p \leq 0.05$).

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+Cd	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+Cd	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+Zn	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+Zn	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+Cd	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+Cd	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 ± 0.959a
	Non-AM+Zn	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+Zn	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+Cd	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+Cd	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+Zn	1.983 ± 0.402^{e}	0.263 ± 0.006^{e}	64.718 ± 9.015^{d}	1.454 ± 0.097^{c}	0.035 ± 0.009d	97.834 ± 0.756^{a}
	AM+Zn	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+Cd	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+Cd	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+Zn	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+Zn	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}

Table 7: Fresh weight, dry weight and moisture content % in shoot and root of non-AM and AM *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Values are expressed as mean ± SE of three independent experiments. Different alphabetical letters indicates significant difference between treatments (Duncan's test, $p \leq 0.05$).

Days	Treatments	Shoot			Root		
		Fresh weight (g)	Dry weight (g)	Moisture content (%)	Fresh weight (g)	Dry weight (g)	Moisture content (%)
0 d	Non-AM	1.731 ± 0.087^{b}	0.45 ± 0.023^{b}	80 .25± 4^{b}	0.81 ± 0.041^{c}	0.11 ± 0.006^{c}	86.42 ± 4.321^{a}
	AM	2.022 ± 0.101^{a}	0.65 ± 0.033^{a}	84 .25± 4.2^{a}	1.45 ± 0.073^{b}	0.23 ± 0.012^{a}	84.138 ± 4.207^{b}
	Non-AM+Cd	1.825 ± 0.091^{b}	0.475 ± 0.024^{b}	81.123 ± 4.05^{b}	0.7 ± 0.035^{d}	0.12 ± 0.006^{c}	85 ± 4.25^{b}
	AM+Cd	2.456 ± 0.123^{a}	0.685 ± 0.034^{a}	86 ± 4.3^{a}	1.41 ± 0.071^{b}	0.25 ± 0.013^{a}	87.654 ± 4.383^{a}
	Non-AM+Zn	1.795 ± 0.09^{b}	0.415 ± 0.021^{b}	79 ± 3.95^{b}	0.88 ± 0.044^{c}	0.11 ± 0.005^{c}	84.615 ± 4.231^{b}
	AM+Zn	2.123 ± 0.106^{a}	0.655 ± 0.033^{a}	85 ± 4.25^{a}	1.84 ± 0.092^{c}	0.186 ± 0.009^{b}	85.884 ± 4.294^{b}
4 d	Non-AM	1.83 ± 0.092^{d}	0.4 ± 0.02^{c}	79.656 ± 3.983^{b}	0.801 ± 0.04^{c}	0.11 ± 0.006^{b}	86.42 ± 4.321^{a}
	AM	2.44 ± 0.122^{b}	0.61 ± 0.031^{b}	87.611 ± 4.381^{a}	1.55 ± 0.078^{b}	0.23 ± 0.012^{a}	85.138 ± 4.257^{b}
	Non-AM+Cd	1.58 ± 0.079^{e}	0.35 ± 0.018^{d}	74.129 ± 3.706^{c}	0.77 ± 0.039^{d}	0.12 ± 0.006^{b}	85 ± 4.25^{b}
	AM+Cd	1.31 ± 0.066^{f}	0.45 ± 0.023^{c}	74.785 ± 3.739^{c}	1.51 ± 0.076^{b}	0.25 ± 0.013^{a}	87.654 ± 4.383^{a}
	Non-AM+Zn	2.07 ± 0.104^{c}	0.62 ± 0.031^{b}	70.854 ± 3.543^{d}	0.78 ± 0.039^{d}	0.12 ± 0.006^{b}	84.615 ± 4.231^{b}
	AM+Zn	2.66 ± 0.133^{a}	0.79 ± 0.04^{a}	72.334 ± 3.617^{d}	1.72 ± 0.086^{a}	0.26 ± 0.013^{a}	84.884 ± 4.244^{b}
8 d	Non-AM	1.932 ± 0.097^{d}	0.5 ± 0.025^{c}	78.142 ± 3.907^{a}	0.81 ± 0.041^{d}	0.11 ± 0.006^{bc}	86.42 ± 4.321^{ab}
	AM	2.225 ± 0.111^{b}	0.65 ± 0.033^{b}	79 ± 3.85^{a}	1.45 ± 0.073^{b}	0.23 ± 0.012^{a}	84.138 ± 4.207^{b}
	Non-AM+Cd	1.46 ± 0.073^{e}	0.4 ± 0.02^{d}	65.649 ± 3.282^{c}	0.6 ± 0.03^{e}	0.09 ± 0.005^{c}	85 ± 4.25^{b}
	AM+Cd	1.32 ± 0.066^{f}	0.42 ± 0.021^{d}	77.848 ± 3.892^{a}	0.81 ± 0.041^{d}	0.1 ± 0.005^{bc}	87.654 ± 4.383^{a}
	Non-AM+Zn	2.05 ± 0.103^{c}	0.59 ± 0.03^{c}	70.048 ± 3.502^{b}	0.68 ± 0.034^{e}	0.09 ± 0.004^{c}	88.615 ± 4.431^{a}
	AM+Zn	2.56 ± 0.128^{a}	0.75 ± 0.038^{a}	70.301 ± 3.515^{b}	0.98 ± 0.049^{c}	0.126 ± 0.006^{b}	88.884 ± 4.444^{a}
12 d	Non-AM	1.932 ± 0.097^{c}	0.5 ± 0.025^{d}	78.142 ± 3.907^{a}	0.801 ± 0.04^{b}	0.11 ± 0.006^{c}	86.42 ± 4.321^{c}
	AM	2.225 ± 0.111^{a}	0.65 ± 0.033^{b}	80 ± 4^{a}	1.55 ± 0.078^{a}	0.23 ± 0.012^{a}	85.138 ± 4.257^{d}
	Non-AM+Cd	1.06 ± 0.053^{e}	0.39 ± 0.02^{e}	65.649 ± 3.282^{c}	0.77 ± 0.039^{c}	0.05 ± 0.003^{e}	96 ± 4.8^{a}
	AM+Cd	1.32 ± 0.066^{f}	0.42 ± 0.021^{d}	77.848 ± 3.892^{a}	1.51 ± 0.076^{a}	0.25 ± 0.013^{a}	87.654 ± 4.383^{c}
	Non-AM+Zn	1.8 ± 0.09^{c}	0.448 ± 0.027^{c}	70.048 ± 3.502^{b}	0.76 ± 0.038^{c}	0.08 ± 0.004^{d}	86.615 ± 4.331^{c}
	AM+Zn	2.06 ± 0.103^{b}	0.685 ± 0.034^{a}	70.301 ± 3.515^{b}	1.12 ± 0.056^{bb}	0.16 ± 0.008^{b}	91.884 ± 4.594^{b}

Table 8: Variations in the root volume of non-AM and AM plants of *Z. mays* and *O. sativa* on exposure to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Values are expressed as mean ± SE of three independent experiments. Different alphabetical letters indicates significant difference between treatments (Duncan's test, $p \leq 0.05$).

	Treatments	Root volume (mL)			
		0 d	4 d	8 d	12 d
Z. mays	Non-AM	4.20 ± 0.567^{c}	5.83 ± 0.690^{c}	5.93 ± 0.600^{d}	5.83 ± 0.620^{c}
	AM	11.25 ± 0.577^{a}	10.25 ± 0.577^{a}	10.20 ± 0.557^{b}	11 ± 0.507^{a}
	Non-AM+Cd	5.83 ± 0.600^{b}	4.83 ± 0.726^{c}	3.83 ± 0.726^{e}	3.23 ± 0.763^{d}
	AM+Cd	11.10 ± 0.577^{a}	8.5 ± 0.288^{b}	8.16 ± 0.126^{c}	8.67 ± 0.166^{b}
	Non-AM+Zn	6.01 ± 0.377^{b}	4.25 ± 0.353^{c}	2.76 ± 0.145^{f}	3.26 ± 0.371^{d}
	AM+Zn	11.33 ± 1.20^{a}	11.58 ± 0.557^{a}	10.66 ± 0.666^{a}	10.76 ± 0.646^{a}
O. sativa	Non-AM	2 ± 0.09^{b}	2.1 ± 0.094^{c}	2.2 ± 0.099^{b}	2 ± 0.09^{c}
	AM	4.5 ± 0.18^{a}	4 ± 0.15^{a}	4.2 ± 0.18^{a}	5 ± 0.16^{a}
	Non-AM+Cd	2.1 ± 0.094^{b}	1.5 ± 0.067^{d}	1 ± 0.045^{d}	1 ± 0.045^{d}
	AM+Cd	4.2 ± 0.189^{a}	3.9 ± 0.155^{b}	2 ± 0.09^{b}	2 ± 0.09^{c}
	Non-AM+Zn	2.5 ± 0.125^{b}	1.8 ± 0.081^{d}	1.5 ± 0.067^{c}	1.8 ± 0.081^{cd}
	AM+Zn	4.5 ± 0.2025^{a}	4.2 ± 0.18^{a}	4 ± 0.18^{a}	3.8 ± 0.20^{b}

Table 9: Modifications in the different JIP parameters in non-AM and AM plants A) *Z. mays* and B) *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Values are expressed as mean ± SE of three independent experiments. Different alphabetical letters indicates significant difference between treatments (Duncan's test, $p \leq 0.05$).

Treatments	*Z. mays*			*O. sativa*		
	Fv/Fm (a.u.)	**PI_{TOTAL} (a.u.)**	**DF_{TOTAL} (a.u.)**	**Fv/Fm (a.u.)**	**PI_{TOTAL} (a.u.)**	**DF_{TOTAL} (a.u.)**
Non-AM	0.786 ± 0.039^{a}	0.2969 ± 0.015^{b}	-0.5274 ± 0.026^{c}	0.833 ± 0.042^{a}	2.733 ± 0.137^{a}	-0.005 ± 0.001^{a}
AM	0.773 ± 0.039^{a}	0.5424 ± 0.027^{a}	-0.2657 ± 0.013^{b}	0.829 ± 0.041^{a}	2.456 ± 0.123^{b}	-0.035 ± 0.002^{b}
Non-AM+Cd	0.597 ± 0.03^{c}	0.0956 ± 0.005^{e}	-1.0197 ± 0.051^{f}	0.735 ± 0.037^{c}	0.936 ± 0.047^{e}	-0.628 ± 0.031^{f}
AM+Cd	0.5435 ± 0.027^{c}	0.15385 ± 0.008^{d}	-0.056 ± 0.003^{a}	0.83 ± 0.042^{a}	1.944 ± 0.097^{c}	-0.358 ± 0.018^{d}
Non-AM+Zn	0.71 ± 0.036^{b}	0.2104 ± 0.011^{c}	-0.6771 ± 0.034^{d}	0.804 ± 0.04^{b}	1.245 ± 0.06^{d}	-0.563 ± 0.028^{e}
AM+Zn	0.712 ± 0.036^{b}	0.1892 ± 0.009^{c}	-0.733 ± 0.037^{e}	0.83 ± 0.041^{a}	2.431 ± 0.122^{b}	-0.216 ± 0.011^{c}

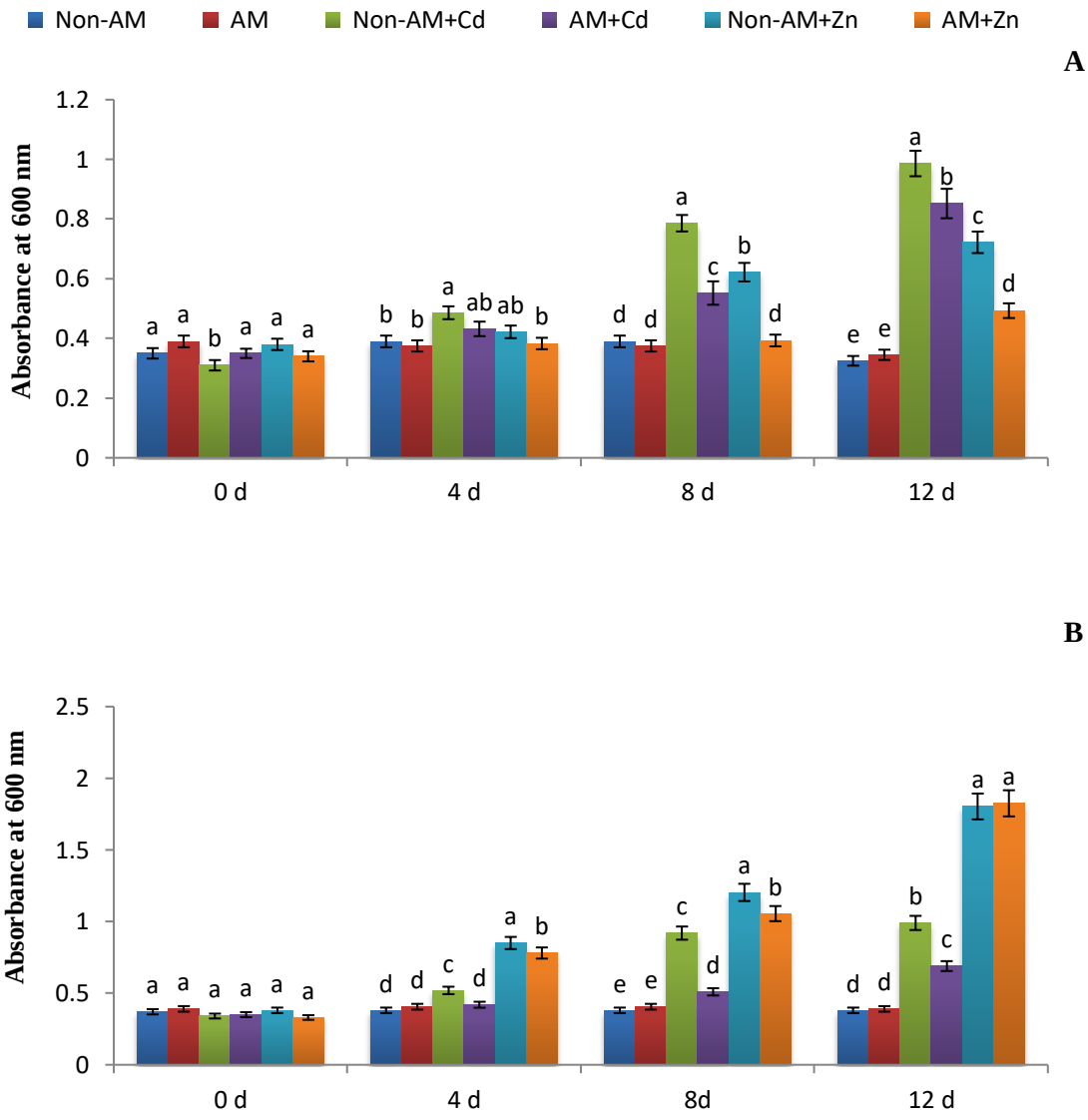

Figure 13: Intensity of root cell death (as per uptake of Evans blue stain) in non-AM and AM plants A) *Z. mays* and B) *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

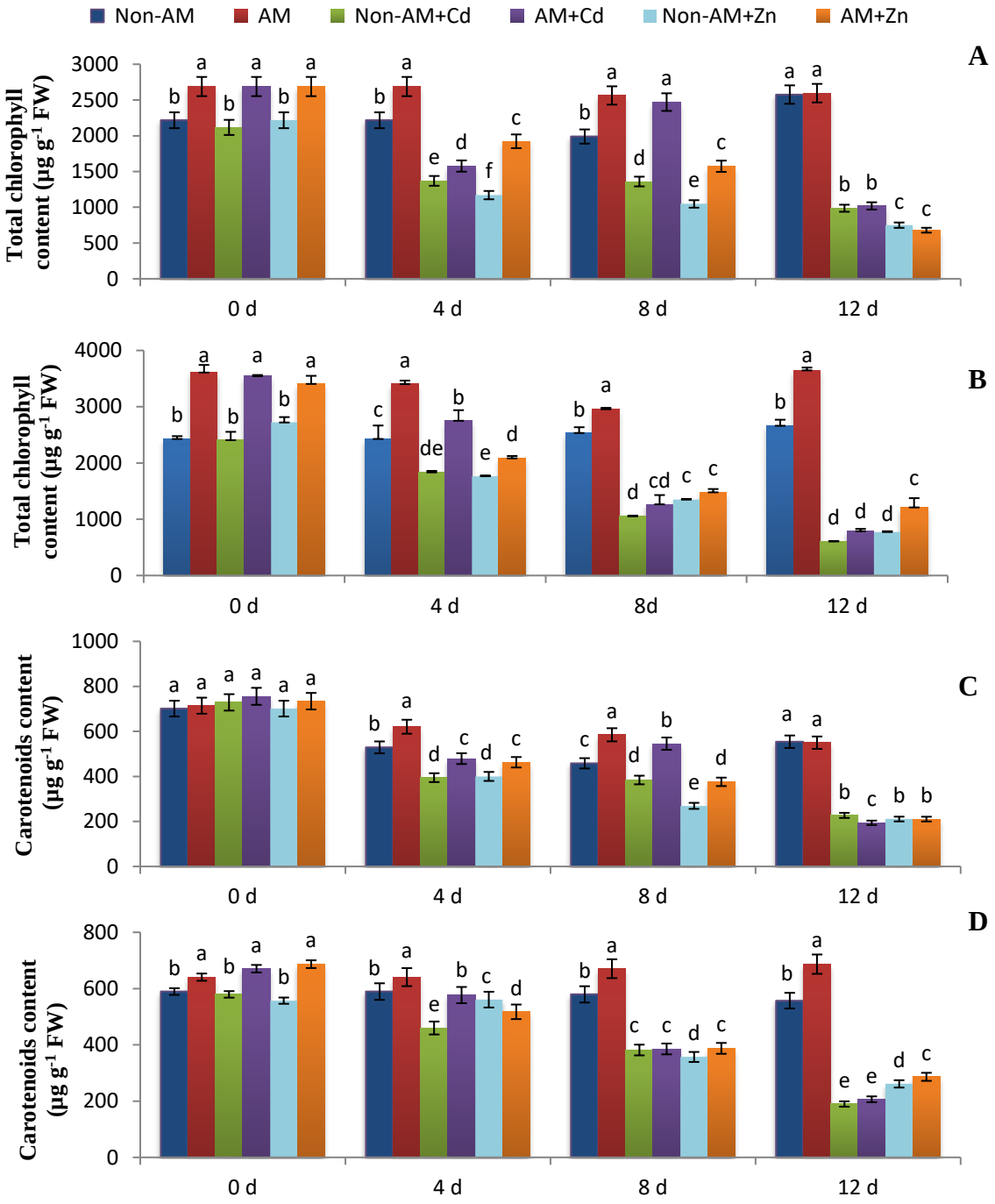

Figure 14: The photosynthetic pigments of non-AM and AM plants; total chlorophyll content of A) *Z. mays* and B) *O. sativa*; carotenoids content of C) *Z. mays* and D) *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

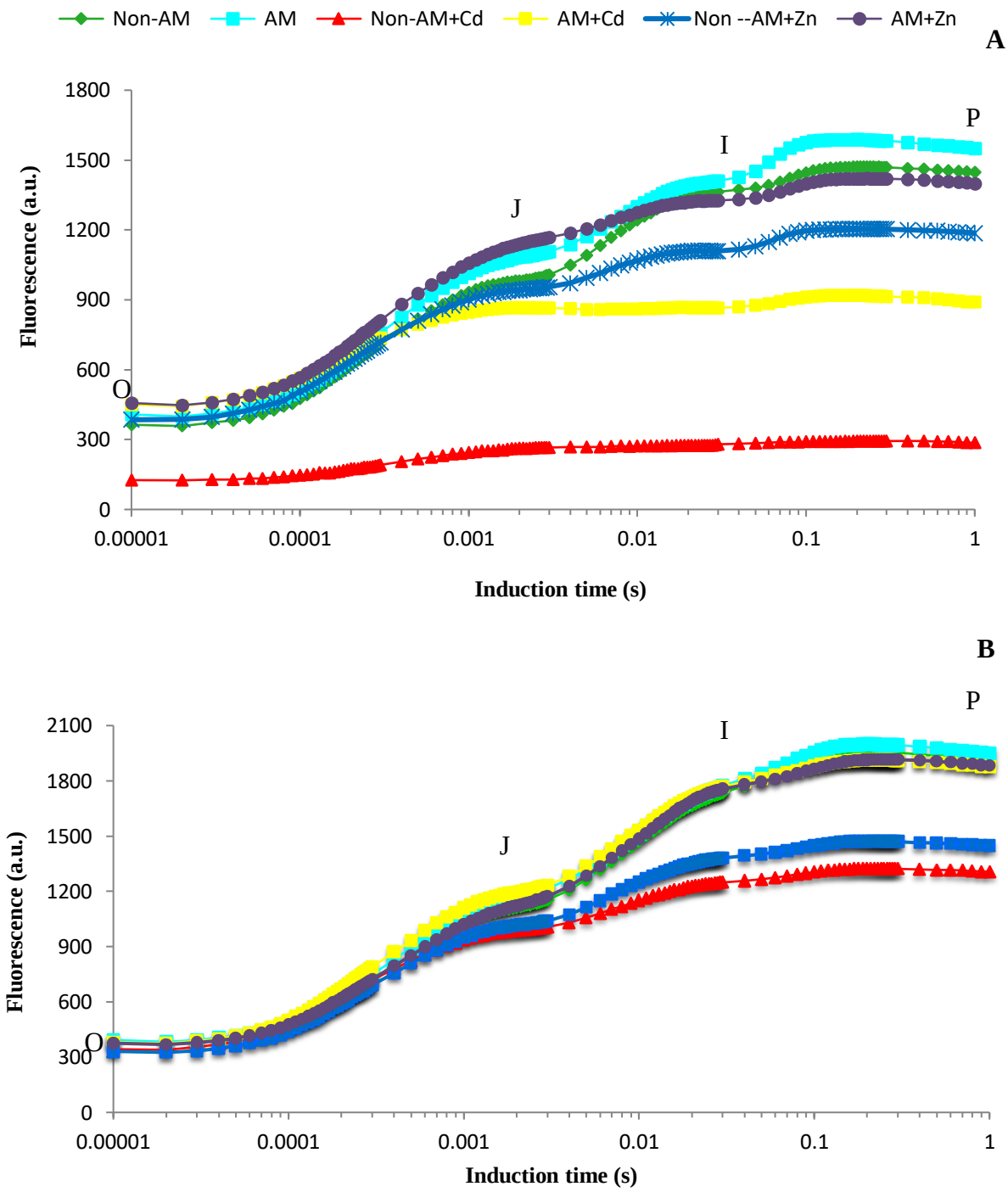

Figure 15: Chlorophyll *a* fluorescence transient curves of non-AM and AM A) *Z. mays* and B) *O. sativa* exposed to 8 d of Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses.

Under Cd stress, ABS/CS_M reduced to 80 and 37% in non-AM and AM *Z. mays* plants respectively, but the reduction was negligible in non-AM and AM plants exposed to Zn stress. In *O. sativa* plants, ABS/CS_M reduced to 32 and 24% in non-AM plants exposed to Cd and Zn stresses respectively, but the reduction was insignificant in AM plants. TRo/CS_M was reduced in Cd treated by 84 and 56% in non-AM and AM *Z. mays* plants respectively. In the case of *O. sativa*, although a reduction in TRo/CS_M was observed in non-AM plants exposed to Cd (37%) and Zn (27%) stresses, it was lower in AM plants exposed to Cd and Zn stresses. Similar responses were observed in the case of ETo/CS_M, in non-AM and AM *Z. mays* plants the reduction was up to 89-93% on exposure to Cd stress. Under Zn stress, 42-46% of reduction in ETo/CS_M was observed in non-AM and AM *Z. mays* plants. In the case of *O. sativa* plants, 60 and 46% of reduction in ETo/CS_M was observed in non-AM plants exposed to Cd and Zn stresses respectively. But an insignificant reduction was observed in AM plants under both the stresses (Fig. 17 and 18).

Dissipation energy *i.e.* DIo/CS_M was drastically reduced in non-AM *Z. mays* plants exposed to Cd stress (Fig. 17). The DIo/CS_M was increased in Cd treated AM plants (33%) as well as in Zn treated non-AM (10%) and AM plants (30%). In *O. sativa* plants, the reduction was insignificant in non-AM and AM plants exposed to Cd and Zn stresses as compared to the control. The closure of reaction centers was represented as dark circles and it was maximum in non-AM *Z. mays* leaves exposed to Cd stress as compared to other treatments (Fig. 17C).

The specific membrane model indicates the response of active (Q_A-reducing PSII) reaction centers towards metal stresses and the parameters recorded were ABS/RC, TRo/RC, ETo/RC and DIo/RC. ABS/RC increased in Cd treated non-AM (10%) and AM (70%) *Z. mays* plants, but under Zn stress insignificant increase was observed in non-AM and AM plants. In the

case of *O. sativa*, the increase in ABS/RC was 55 and 17% in non-AM plants exposed to Cd and Zn stresses respectively. In AM plants, an insignificant increase in ABS/RC was observed due to the exposure to metal stress (Fig. 19 and 20).

In *Z. mays* plants, TRo/RC was decreased in non-AM plants exposed to Cd, but it insignificantly enhanced in Cd treated AM plants and Zn treated non-AM and AM plants. TRo/RC was increased to 27 and 13% in non-AM *O. sativa* plants exposed to Cd and Zn stresses respectively. But Zn stress did not induce any important and notable changes in TRo/RC (Fig. 19 and 20).

In the case of ETo/RC, the metal induced reduction was very prominent. In *Z. mays*, 65-70% of reduction in ETo/RC was observed in non-AM and AM plants exposed to Cd stress. But, the reduction was only 24-40% in non-AM and AM plants exposed to Zn stress. In *O. sativa* plants, the reduction was 34 and 7% in Cd treated non-AM and AM plants respectively. Under Zn stress, the reduction of the same parameters was lesser and it was 19 and 2% in non-AM and AM plants respectively (Fig. 19 and 20).

DIo/RC drastically increased under Cd and Zn toxicity. In *Z. mays*, the increase was 108 and 265% in Cd exposed non-AM and AM plants respectively. But there was only 55 and 36% increase in non-AM and AM plants exposed to Zn stress. In *O. sativa*, the increase of DIo/RC in non-AM plants subjected to Cd and Zn stresses were 194 and 41% respectively, as compared to the control. However, the increase was negligible in AM plants (Fig. 19 and 20).

4.3.5.3 Photosystem (PS) I and II activities

Photosystem I and II activities of *Z. mays* and *O. sativa* were severely reduced by the toxic effects of Cd and Zn, and it increased with increase in the days of metal exposure (Fig. 21). The reduction in the activity of PSII was

to the extent of 46-61% in non-AM plants of *Z. mays* exposed to 12 d of Cd and Zn stresses, but the reduction was only 32-34% in AM plants. In the case of non-AM plants of *O. sativa*, the reduction in PSII activity was to the extent of 29-34%, but it was only 14-29% in AM plants on 12 d of imparting Cd and Zn stresses (Fig. 21A and B). A significant positive correlation observed between total chlorophyll content and PSII activity of non-AM ($r = 0.796$, $p \leq 0.05$) and AM ($r = 0.921$, $p \leq 0.01$) plants of *Z. mays* exposed to Cd and Zn stresses (Table 10 and 11). Similarly, in *O. sativa* a strong significant positive correlation observed between total chlorophyll content and PSII activity of non-AM ($r = 0.967$, $p \leq 0.01$) and AM ($r = 0.921$, $p \leq 0.01$) plants exposed to Cd and Zn toxicity (Table 12 and 13).

Similar to the response of PSII activity, PSI activity was also reduced due to influence of Cd and Zn stresses. The reduction in the activity of PSI was 33-50% in non-AM plants of *Z. mays*, at the same time it was only 23-32% in AM plants exposed to Cd and Zn stresses. When *O. sativa* was exposed to Cd stress, the reduction in PSI activity was 23 and 14% in non-AM and AM plants respectively, but it was 25-27% on 12 d of Zn stress (Fig. 21C and D).

4.3.5.4 Leaf micromorphological characters

Leaf micromorphological characters of *Z. mays* were significantly modified during the exposure to Cd and Zn stresses (Fig. 22, 23, 24, and 25). Abaxial stomata were completely closed in non-AM plants and partially closed AM plants exposed to Cd and Zn stresses (Fig. 22 and 23). At the same time, adaxial stomata of non-AM plants completely closed on exposure to Cd stress, whereas insignificant modifications was observed on the adaxial stomata of AM plants under Cd stress. Zinc toxicity induced abnormal opening of stomata in non-AM plants, but it was not observed in AM plants. Abaxial and adaxial surfaces of leaves in non-AM and AM *Z. mays* plants did

not exhibit any other structural modifications under Cd and Zn stresses (Fig. 24 and 25).

Analysis of leaf micromorphological characters of *O. sativa* revealed that the Cd and Zn treated plants significantly variated from that of the control plants (Fig. 26, 27, 28 and 29). Partial closure of adaxial stomata was observed in Cd treated *O. sativa* plants and it was completely closed in Zn treated non-AM and AM plants (Fig. 26). The stomata in the abaxial surface was completely closed in non-AM plants under Cd stress (Fig. 27). The upper leaf surface of non-AM plants exposed Zn developed a circular shaped special structures, which appeared like an opening on exposure to Zn, but similar openings were not observed in AM plants under the same treatment (Fig. 28). At the same time, abaxial surface of non-AM and AM plants did not exhibit this type of structural modifications (Fig. 29).

4.4 Biochemical parameters

4.4.1 Reactive oxygen species (ROS) accumulation and membrane degradation

Cadmium and Zn stresses caused an increase in ROS content, membrane degradation and electrolyte leakage in *Z. mays* and *O. sativa*, however mycorrhization reduced the ROS imbalance in both the plants.

4.4.1.1 Superoxide ($O_2^{\cdot-}$) content

Both Cd and Zn stresses caused increase in the superoxide content in the leaves of non-AM and AM plants of *Z. mays* and *O. sativa* and the accumulation of superoxide content was increased with an increase in days of exposure (Fig. 30A and B). Increase in the superoxide content was 5 and 4 fold in non-AM plants of *Z. mays* under Cd and Zn stresses respectively. But the increase was only 2-3 fold in AM plants exposed to Cd and Zn stresses on

8 d. In the case of Cd and Zn treated non-AM plants of *O. sativa*, the augmentation in accumulation of superoxide was 119-259% and in AM plants the increase was only to the extent of 92-205% on 8 d of stress exposure. Superoxide content in the leaves of non-AM *Z. mays* had a strong significant negative correlation with F_V/F_M ($r = -0.880$, $p \leq 0.01$), but it was insignificant in AM ($r = 0.432$, $p \geq 0.05$) plants exposed to Cd and Zn stresses (Table 10 and 11). Whereas the superoxide content in the leaves of *O. sativa* had an insignificant correlation with F_V/F_M in non-AM ($r = 0.61$, $p \geq 0.05$) and AM ($r = 0.432$, $p \geq 0.05$) plants exposed to Cd and Zn stresses (Table 12 and 13).

Both Cd and Zn stresses caused increase in the superoxide content in the roots of non-AM and AM plants (Fig. 30C and D). In *Z. mays*, the increase was 115-137% in non-AM plants and 46-63% in AM plants exposed to Cd and Zn stresses. In Cd treated non-AM and AM plants of *O. sativa*, the increase in superoxide content was 47 and 31% respectively. But it was negligible in non-AM and AM plants on 8 d of exposure to Zn stress (Fig. 30D).

4.4.1.2 Hydrogen peroxide (H_2O_2) content

Both Cd and Zn stresses lead to the accumulation of H_2O_2 in the leaves of non-AM and AM plants of *Z. mays* and *O. sativa* (Fig. 31A and B). The increase was 50 and 32% in non-AM plants of *Z. mays* exposed to Cd and Zn stresses respectively and the increase was insignificant in AM plants on 4 d of metal exposure. The accumulation of H_2O_2 increased with an increase in the days of exposure. On 8 d of Cd and Zn stresses, the increase in H_2O_2 content was 4-5 fold in non-AM plants, whereas the increase was up to 3-4 fold in AM plants as compared to control. In *O. sativa*, the increase was 32 and 95% in Cd and Zn treated non-AM plants, but the increase was only 13 and 38% in AM plants on 4 d of exposure. On 8 d of imparting Cd and Zn stresses, the increase in H_2O_2 content was 5-6 fold in non-AM plants and 4-5 fold in AM

plants as compared to the control. Hydrogen peroxide content in the leaves of non-AM *Z. mays* plants had a strong significant negative correlation with F_v/F_M (r = -0.878, $p \leq 0.01$), but it was insignificant in AM (r = 0.476, $p \geq 0.05$) plants exposed to Cd and Zn stresses (Table 10 and 11). Whereas the hydrogen peroxide content in the leaves of non-AM (r = -0.146, $p \geq 0.05$) and AM (r = 0.476, $p \geq 0.05$) *O. sativa* had an insignificant correlation with F_v/F_M on exposure to Cd and Zn stresses (Table 12 and 13).

H_2O_2 content was increased in roots of non-AM and AM plants on exposure to Cd and Zn stresses (Fig. 31C and D). In non-AM plants of *Z. mays* the increase was 272 and 139% on 8 d of Cd and Zn stresses respectively, but in AM plants it was only 103 and 54%. In the case of *O. sativa*, the enhancement of H_2O_2 was 364 and 462% in non-AM plants, but the increase was only 310 and 392% in AM plants on 8 d of Cd and Zn stresses.

4.4.1.3 Malondialdehyde (MDA) content

Both Cd and Zn stresses caused MDA accumulation in the leaves of non-AM and AM plants of *Z. mays* and *O. sativa* (Fig. 32A and B). MDA content was increased to 3 and 4 fold in non-AM plants of *Z. mays* under Cd and Zn stresses respectively, but in AM plants 2 and 4 fold increase was observed on 8 d of exposure to these metals. In *O. sativa*, 2 and 4 fold increase of MDA content was observed in non-AM plants under Cd and Zn stresses, but it was only 1.5 and 3 fold in AM plants on 8 d of exposure. MDA content had a strong significant negative correlation with the total chlorophyll content in the leaves of non-AM *Z. mays* plants (r = -0.997, $p \leq 0.01$), but it was insignificant in AM (r = -0.491, $p \geq 0.05$) plants exposed to Cd and Zn stresses. Whereas the MDA content in the leaves of non-AM *O. sativa* plants had an insignificant negative correlation with total chlorophyll content (r = -0.562, $p \geq 0.05$) and AM (r = -0.491, $p \geq 0.05$) on exposure to Cd and Zn

stresses. Moreover, MDA content had a strong significant negative correlation with the activity of PSII in the leaves of non-AM *Z. mays* plants (r = -0.841, $p \leq 0.01$), and it was significant in AM (r = -0.785, $p \geq 0.05$) plants exposed to Cd and Zn stresses (Table 10 and 11). Similarly, in the leaves of non-AM *O. sativa* plants, MDA content had a strong significant negative correlation with the PSII activity (r = -0.693, $p \leq 0.05$), and it was significant in AM (r = -0.785, $p \leq 0.05$) plants exposed to Cd and Zn stresses (Table 12 and 13).

In contrast to that of shoot, MDA content was reduced in roots of non-AM and AM *Z. mays* and *O. sativa* plants on exposure to Cd and Zn stresses (Fig. 32C and D). In *Z. mays* exposed to Cd stress, MDA content was reduced to the extent of 33-40% in non-AM and AM plants whereas, the reduction was insignificant under Zn stress. The reduction of MDA content was 43 and 61% in roots non-AM plants of *O. sativa*, but the reduction was only 21 and 48% in roots of AM plants on 8 d of Cd and Zn stresses.

4.4.1.4 Membrane stability index (MSI)

Both Cd and Zn stresses caused reduction in the membrane stability index of *Z. mays* and *O. sativa* leaves and the impact was increased with an increase in the days of exposure (Fig. 33A and B). MSI of *Z. mays* was reduced to 77 and 89% in non-AM plants exposed to Cd and Zn stresses respectively whereas the reduction was 59 and 70% in AM plants exposed to 12 d of Cd and Zn stresses respectively. In the case of *O. sativa*, the reduction in MSI was to the extent of 21 and 39% on exposure to Cd and Zn stresses respectively, but it was only 12-15% in AM plants.

In roots of *Z. mays*, the reduction of MSI was 90% in non-AM plants, but was insignificant in AM plants exposed to 12 d of Cd and Zn stresses (Fig. 33C and D). The reduction in MSI was 71 and 61% in non-AM plants,

and insignificant in AM plants of *O. sativa* exposed to 12 d of Cd, and Zn stresses.

4.4.1.5 Electrolyte leakage

Electrolyte leakage was increased in *Z. mays* and *O. sativa* by the toxic effects of Cd and Zn stresses, which was further increased with an increase in the days of metal exposure. However, mycorrhization aided in reducing the electrolyte leakage and it reached to a level same as that of the control (Fig. 34A and B). Cadmium and Zn stresses induced increase in the electrolyte leakage was 308 and 236% in non-AM plants of *Z. mays* on 8 d of metal exposure, but it was only 60 and 79% in the leaves of AM plants. In *O. sativa*, the increase in electrolyte leakage was 196 and 237% in the leaves of non-AM plants exposed to 8 d of Cd and Zn stresses respectively. But only 36 and 53% of enhancement in electrolyte leakage was observed in AM plants as compared to the control plants.

In roots of *Z. mays,* the increase in electrolyte leakage was to the extent of 47-57% in non-AM plants, and 29-36% in AM plants exposed to 8 d of Cd and Zn stresses. In *O. sativa,* 51 and 30% of increase in the electrolyte leakage was observed on 8 d of Cd and Zn stresses respectively. But the increase was only 11-17% in AM plants exposed to Cd and Zn stresses (Fig. 34C and D).

4.4.2 Primary metabolites

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.

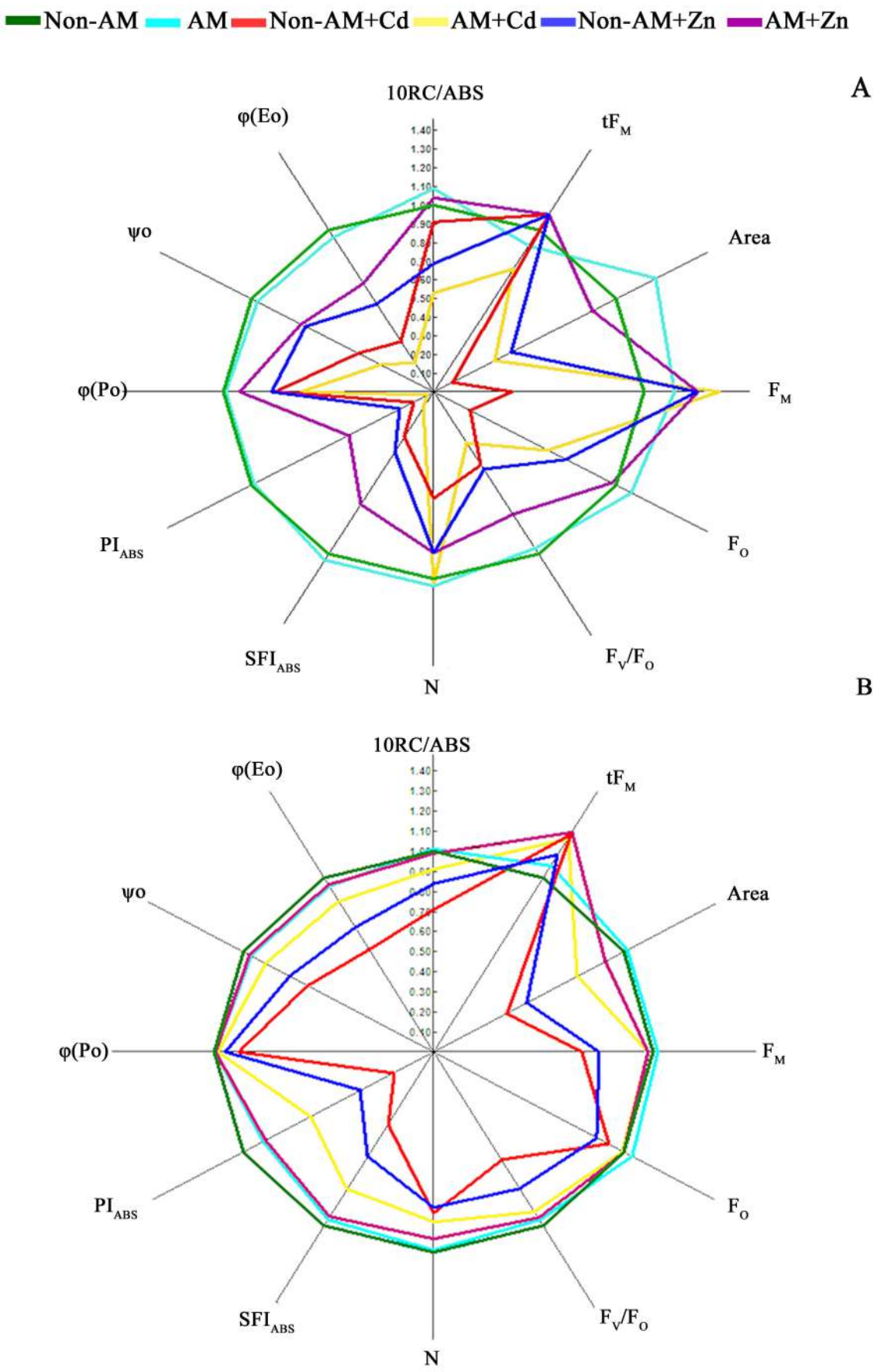

Figure 16: Radar plot of different JIP parameters in non-AM and AM plants A) *Z. mays* and B) *O. sativa* exposed to 8 d of Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses

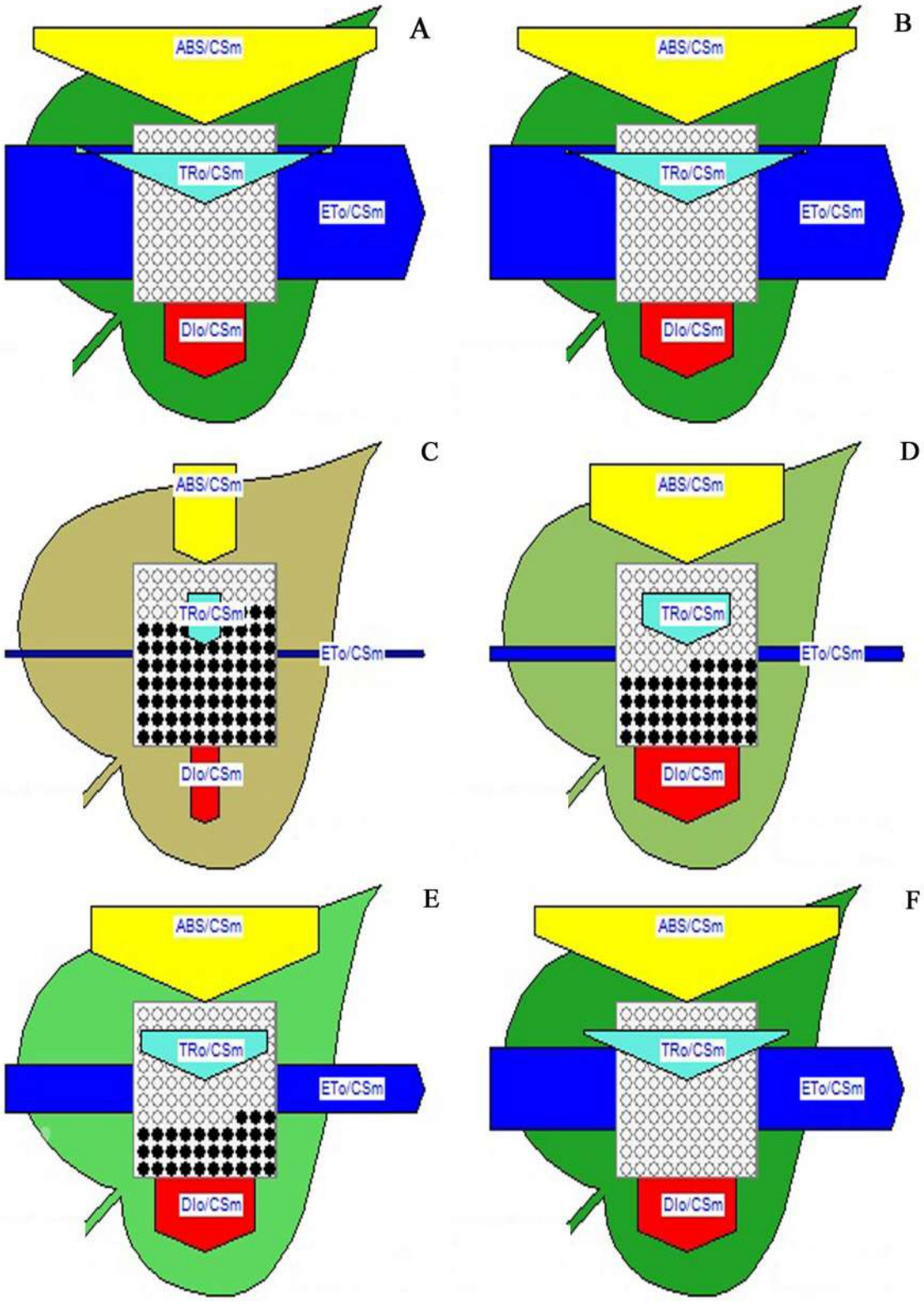

Figure 17: Leaf pipeline model showing the proportion of phenomenological energy flux parameters (calculated per cross section approximated by maximal fluorescence) in non-AM and AM plants of *Z. mays* exposed to 8 d of Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd D) AM+Cd, E) non-AM+Zn and F) AM+Zn.

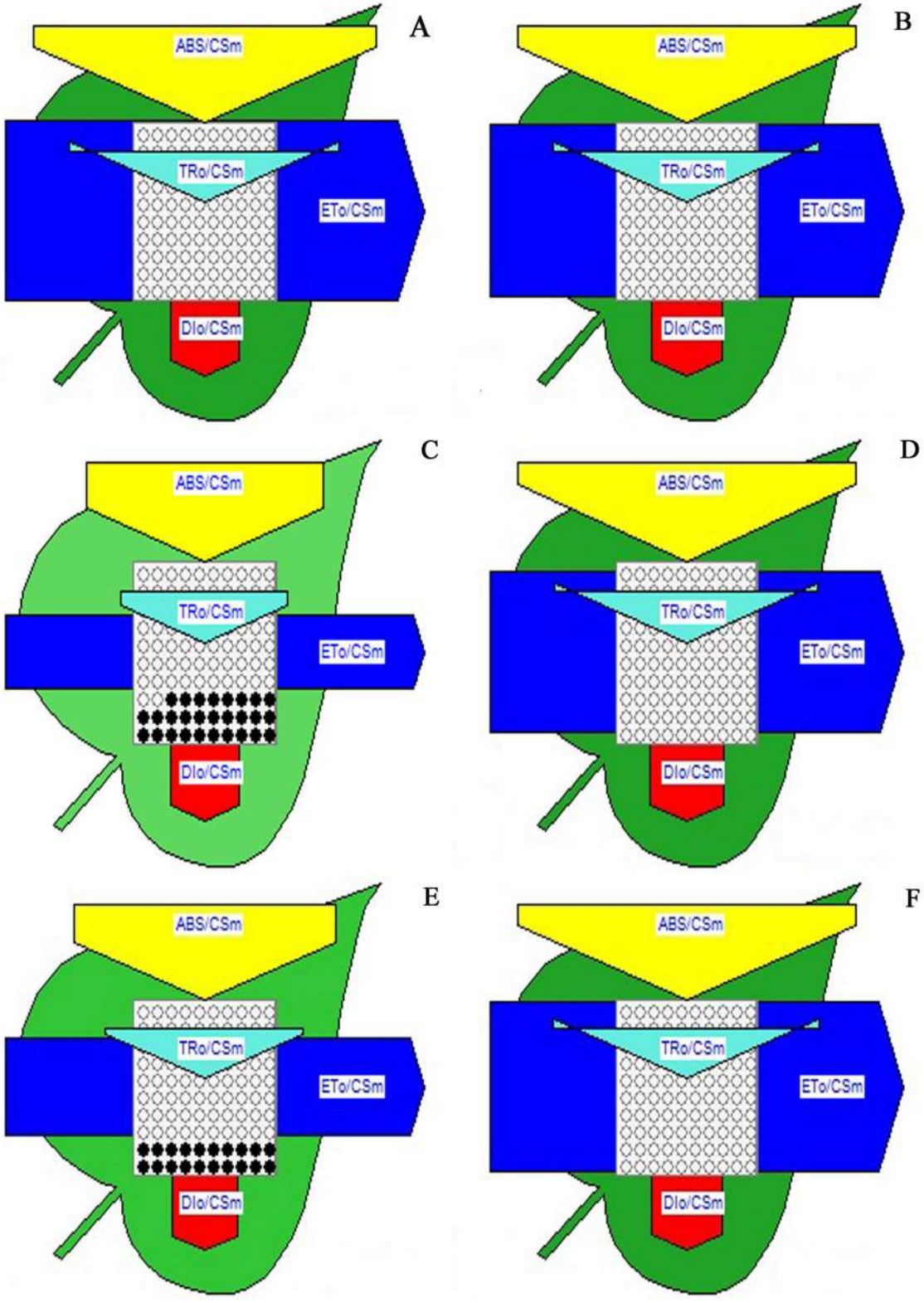

Figure 18: Leaf pipeline model showing the proportion of phenomenological energy flux parameters (calculated per cross section approximated by maximal fluorescence) in non-AM and AM plants of *O. sativa* exposed to 8 d of Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B)AM, C) non-AM+Cd D) AM+Cd, E) non-AM+Zn and F) AM+Zn.

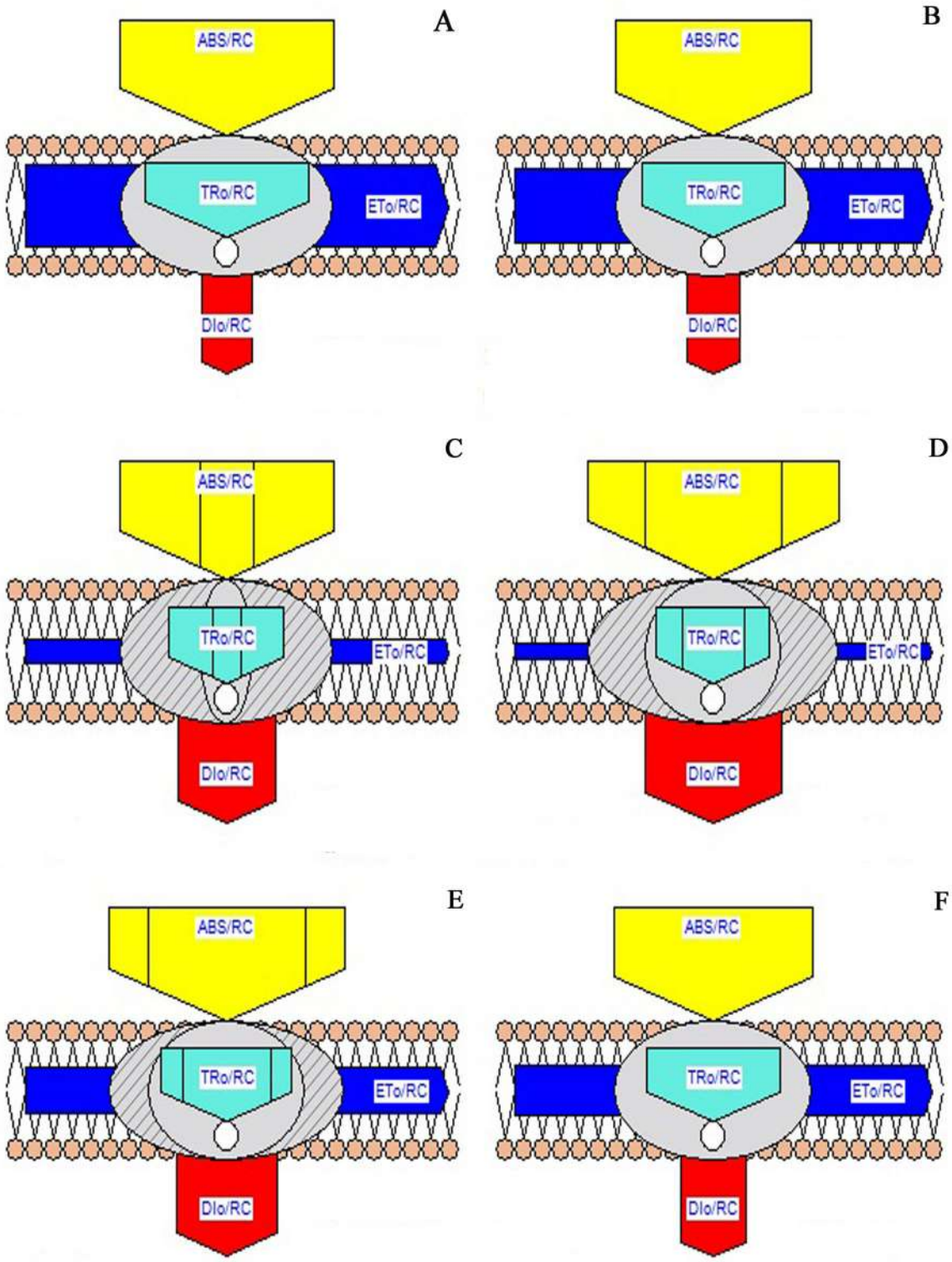

Figure 19: The membrane model representing specific activities expressed per reaction center (RC) in non-AM and AM plants of *Z. mays* exposed to 8 d of Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd D) AM+Cd, E) non-AM+Zn and F) AM+Zn.

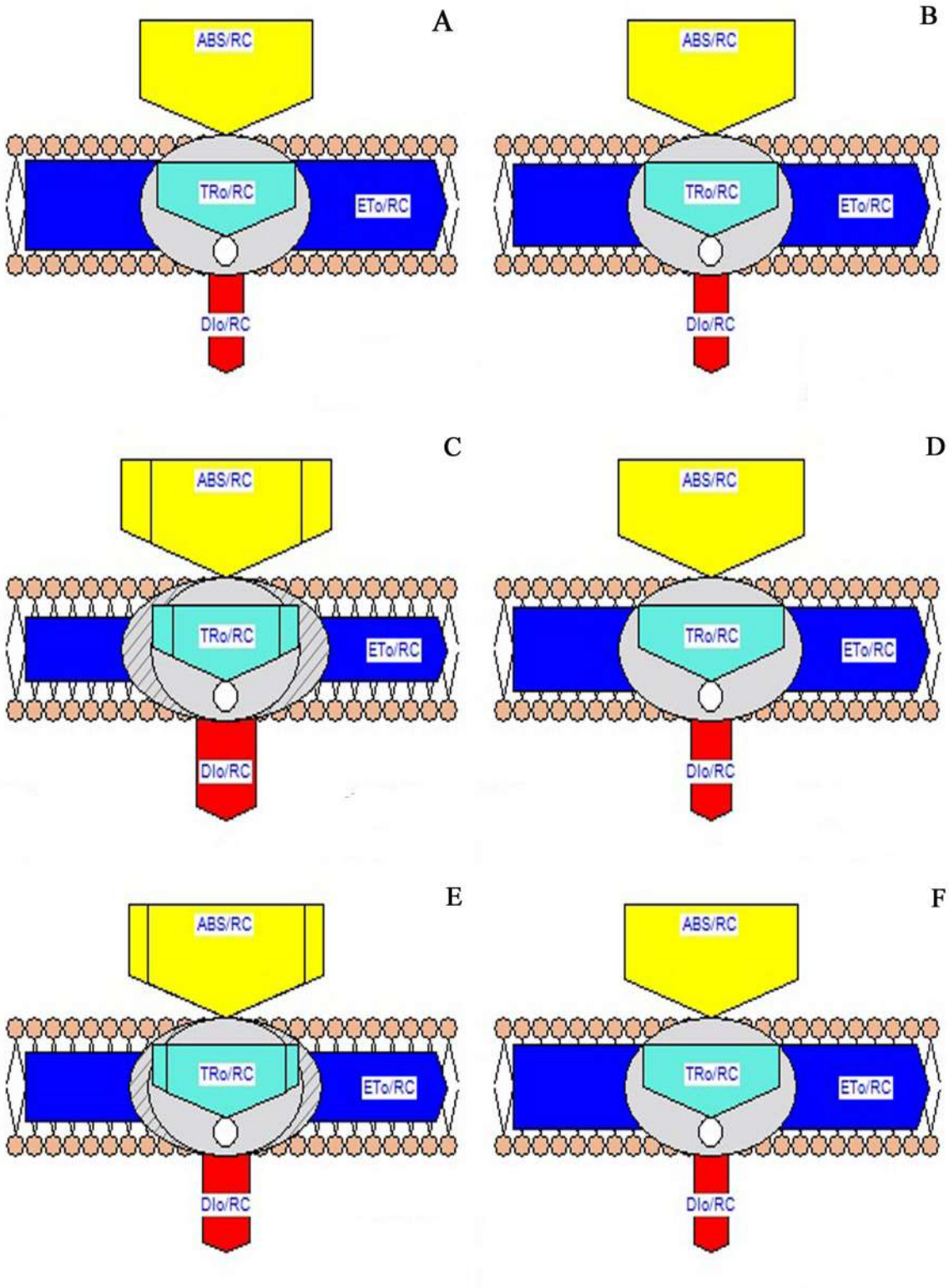

Figure 20: The membrane model representing specific activities expressed per reaction center (RC) in non-AM and AM plants of *O. sativa* exposed to 8 d of Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd D) AM+Cd, E) non-AM+Zn and F) AM+Zn.

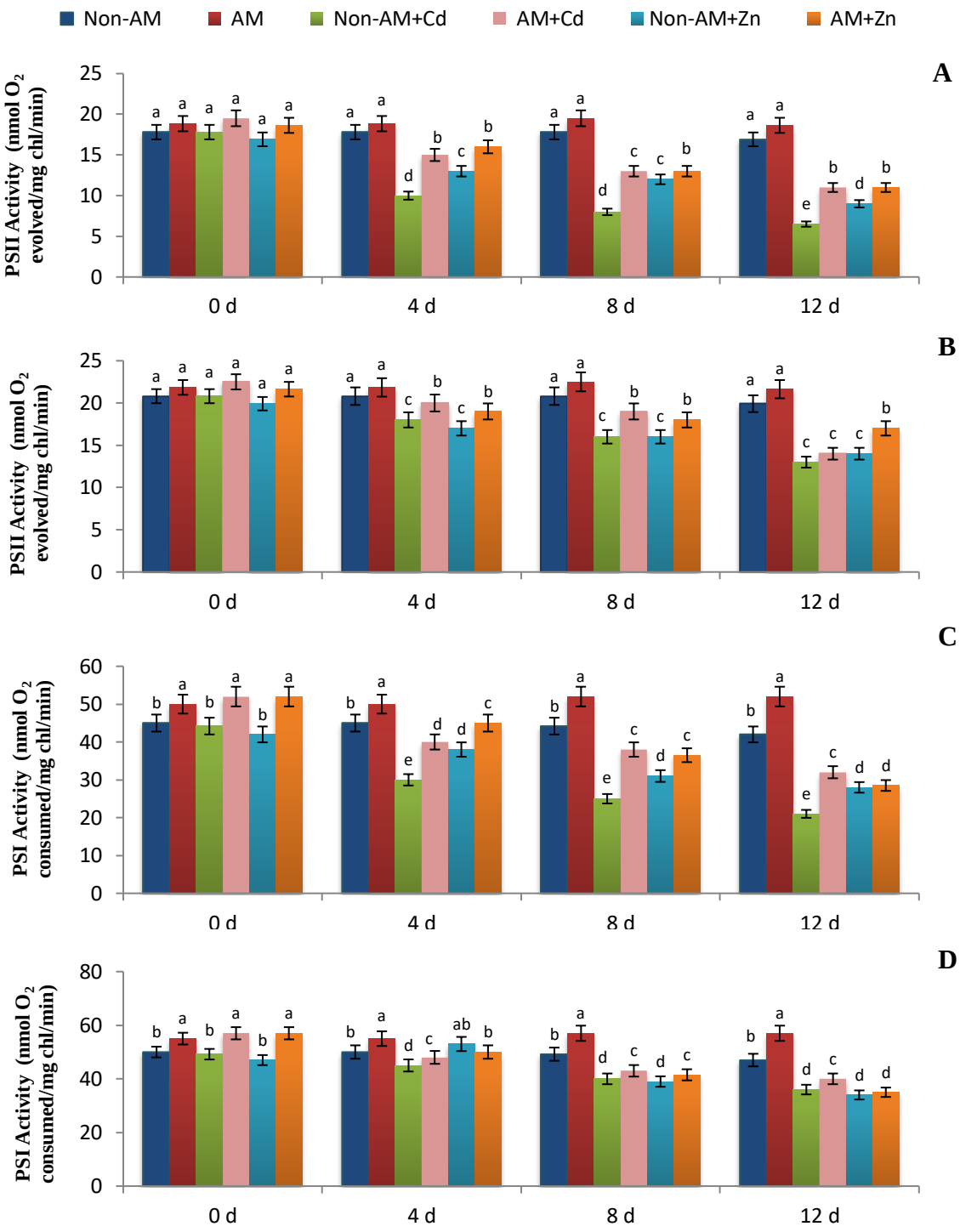

Figure 21: Photosystem activities of non-AM and AM plants; A) PSII activity of *Z. mays*, B) PSII activity of *O. sativa*, C) PSI activity of *Z. mays* and D) PSI activity of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

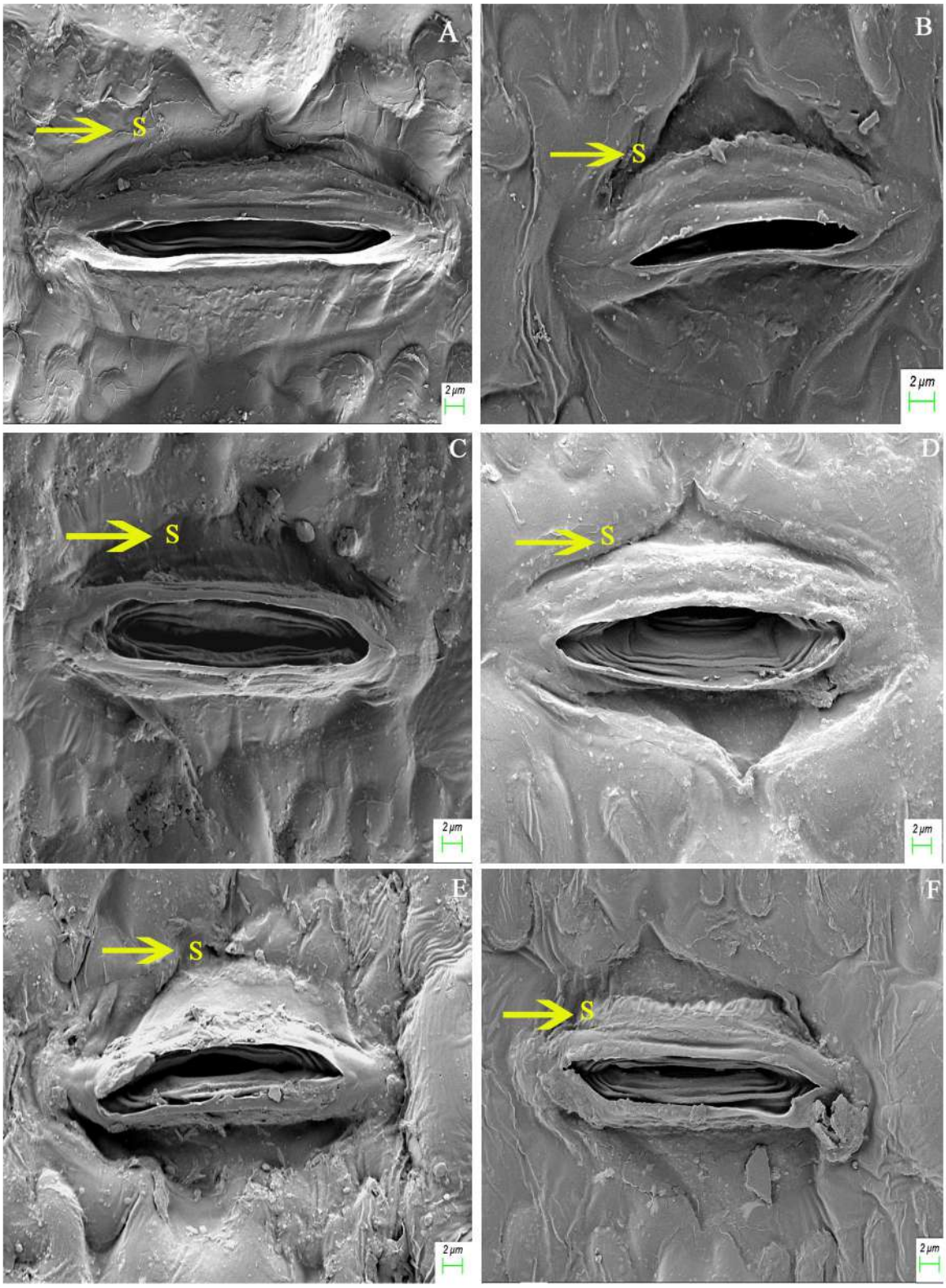

Figure 22: Scanning electron micrographs of adaxial stomata in *Z. mays* associated with mycorrhiza and exposed to Cd (0.45 g kg^{-1}) and Zn (1. 95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd D) AM+Cd, E) non-AM+Zn and F) AM+Zn; where s-stomata.

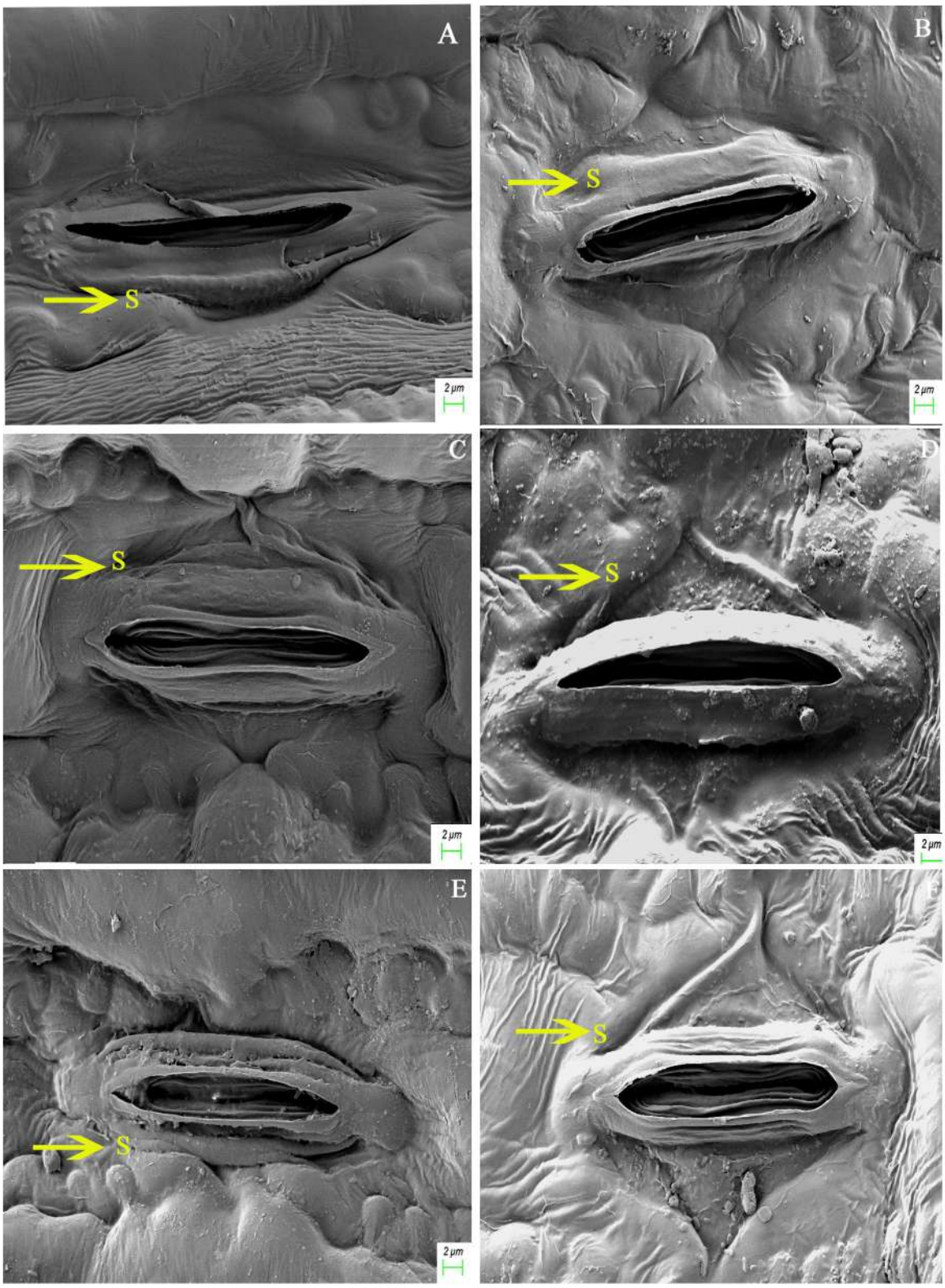

Figure 23: Scanning electron micrographs of abaxial stomata in *Z. mays* associated with mycorrhiza and exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd D) AM+Cd, E) non-AM+Zn and F) AM+Zn; where s-stomata.

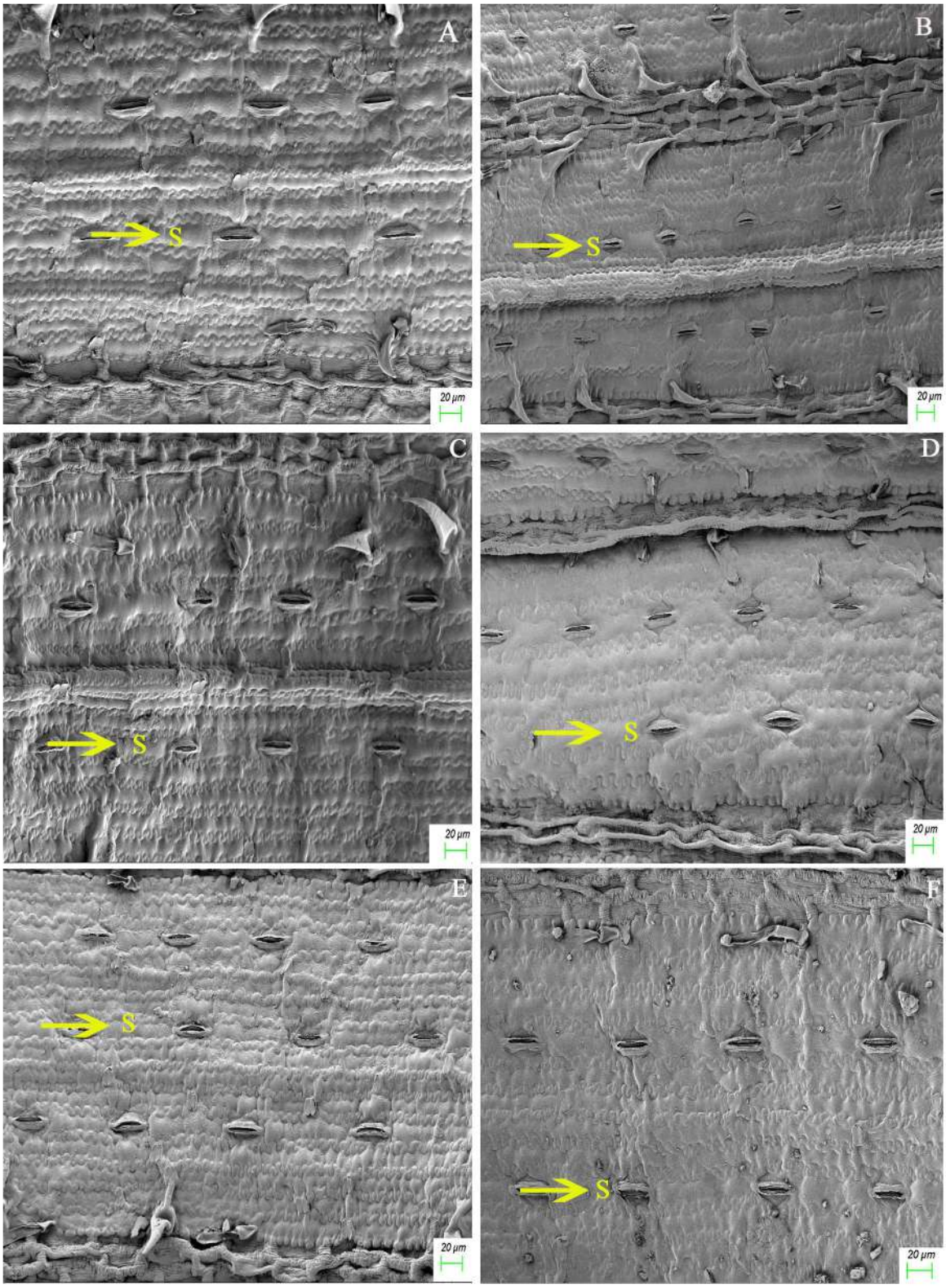

Figure 24: Scanning electron micrographs of adaxial leaf surface in *Z. mays* associated with mycorrhiza and exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd D) AM+Cd, E) non-AM+Zn and F) AM+Zn; where s-stomata.

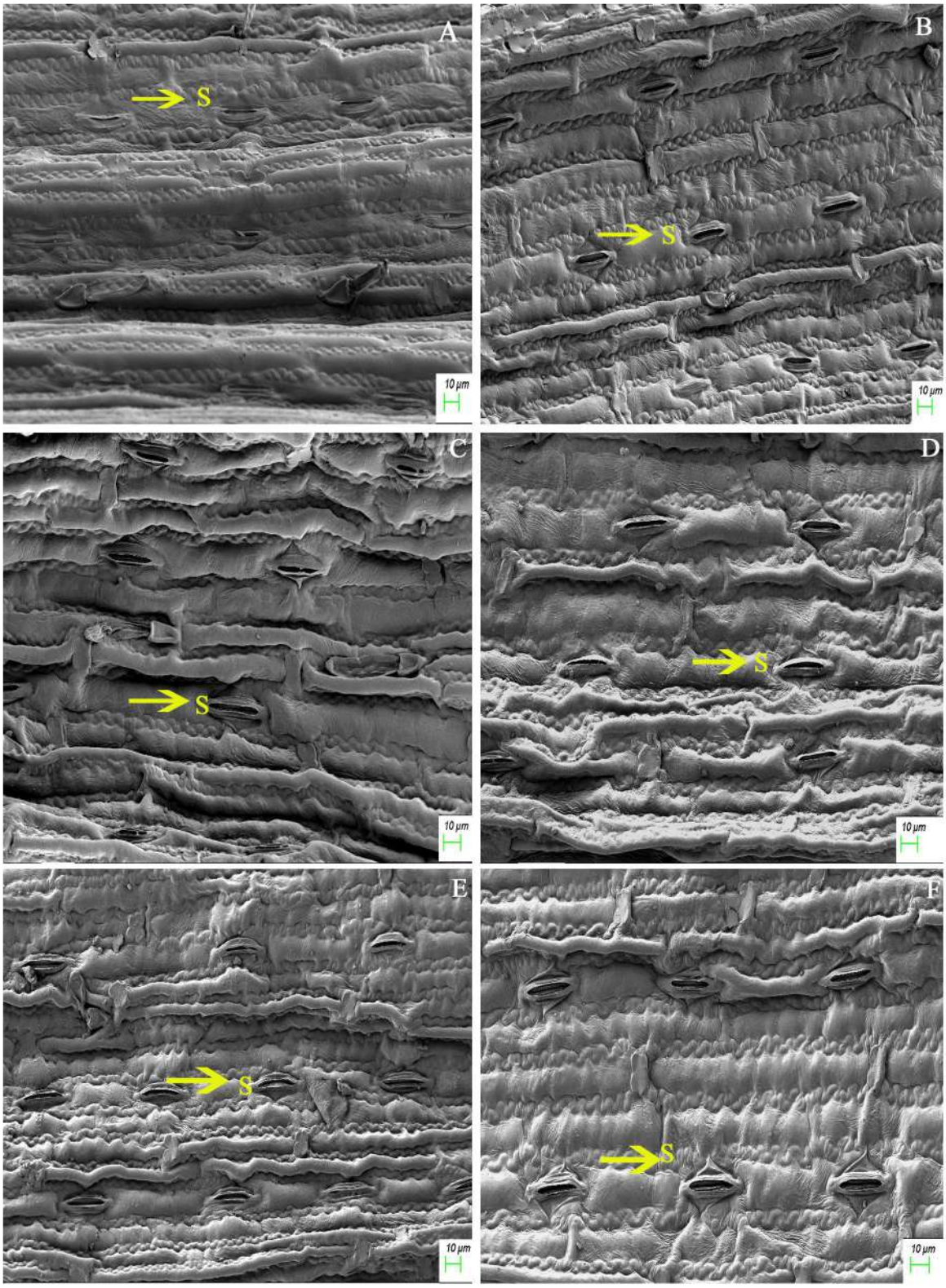

Figure 25: Scanning electron micrographs of abaxial leaf surface in *Z. mays* associated with mycorrhiza and exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd D) AM+Cd, E) non-AM+Zn and F) AM+Zn; where s-stomata.

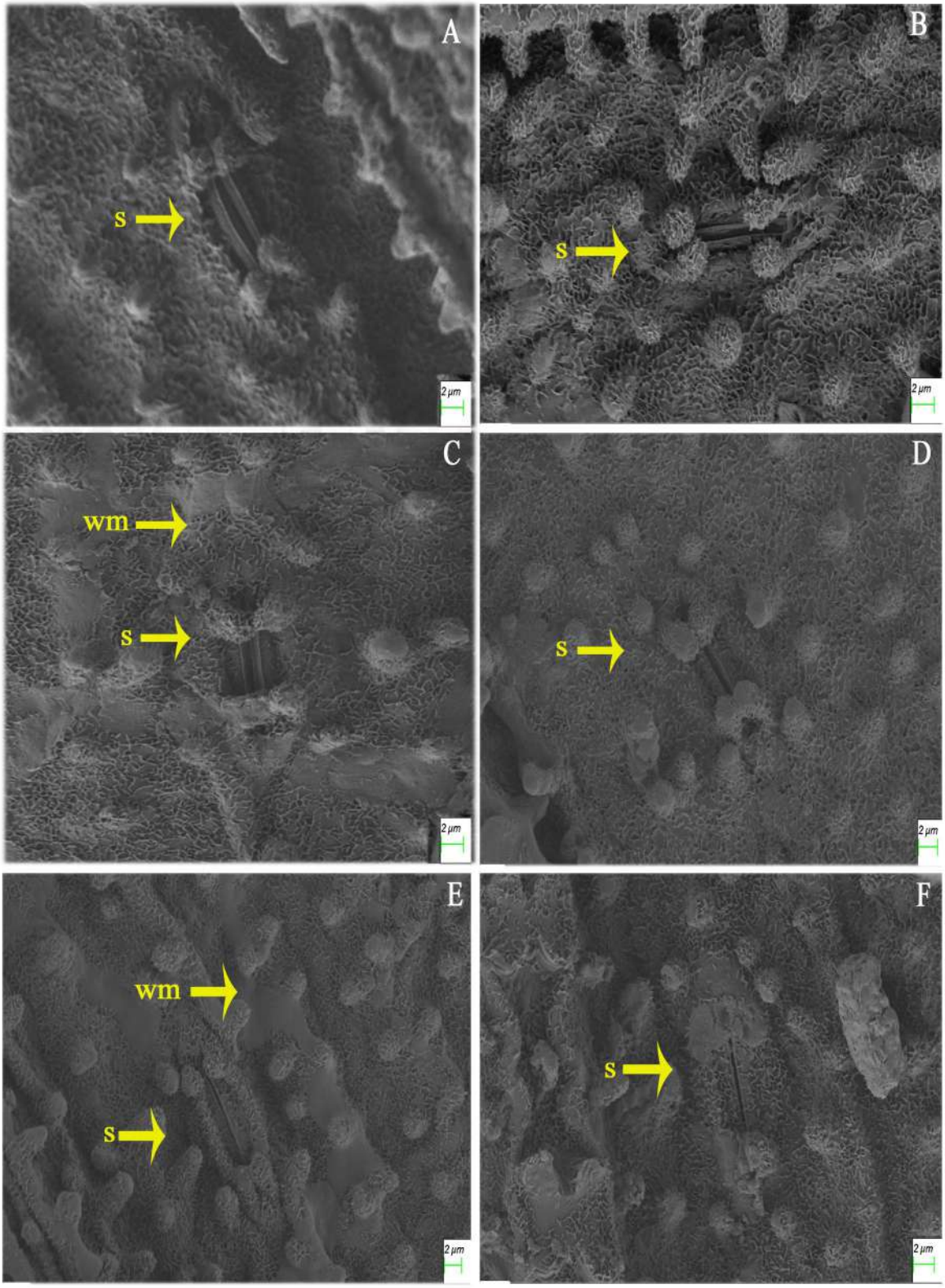

Figure 26: Scanning electron micrographs of adaxial stomata in *O. sativa* associated with mycorrhiza and exposed to Cd (0.45 g kg^{-1}) and Zn (1. 95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd D) AM+Cd, E) non-AM+Zn and F) AM+Zn; where s-stomata and wm-wax melting.

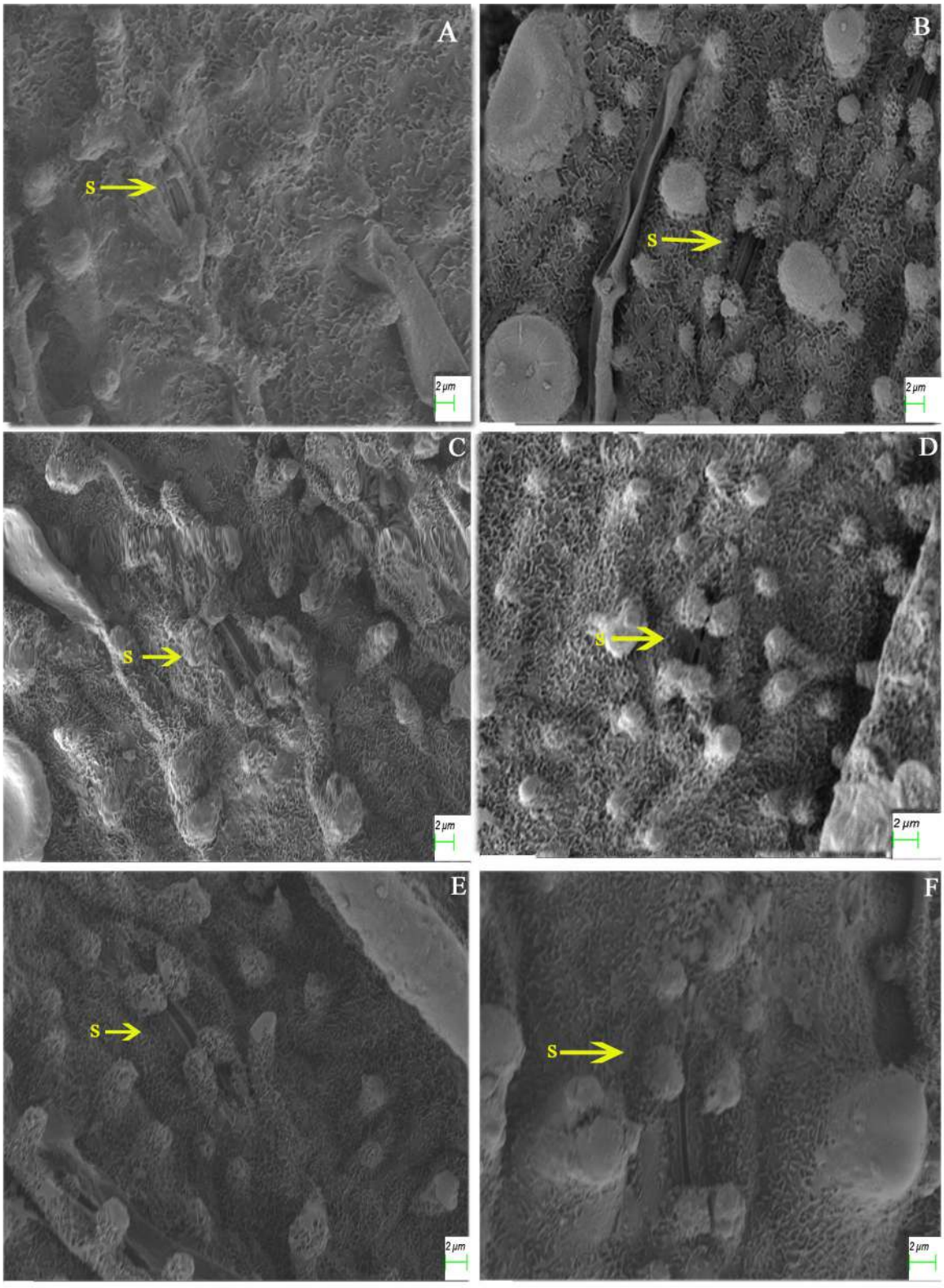

Figure 27: Scanning electron micrographs of abaxial stomata in *O. sativa* associated with mycorrhiza and exposed to Cd (0.45 g kg^{-1}) and Zn (1. 95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd D) AM+Cd, E) non-AM+Zn and F) AM+Zn; where s-stomata.

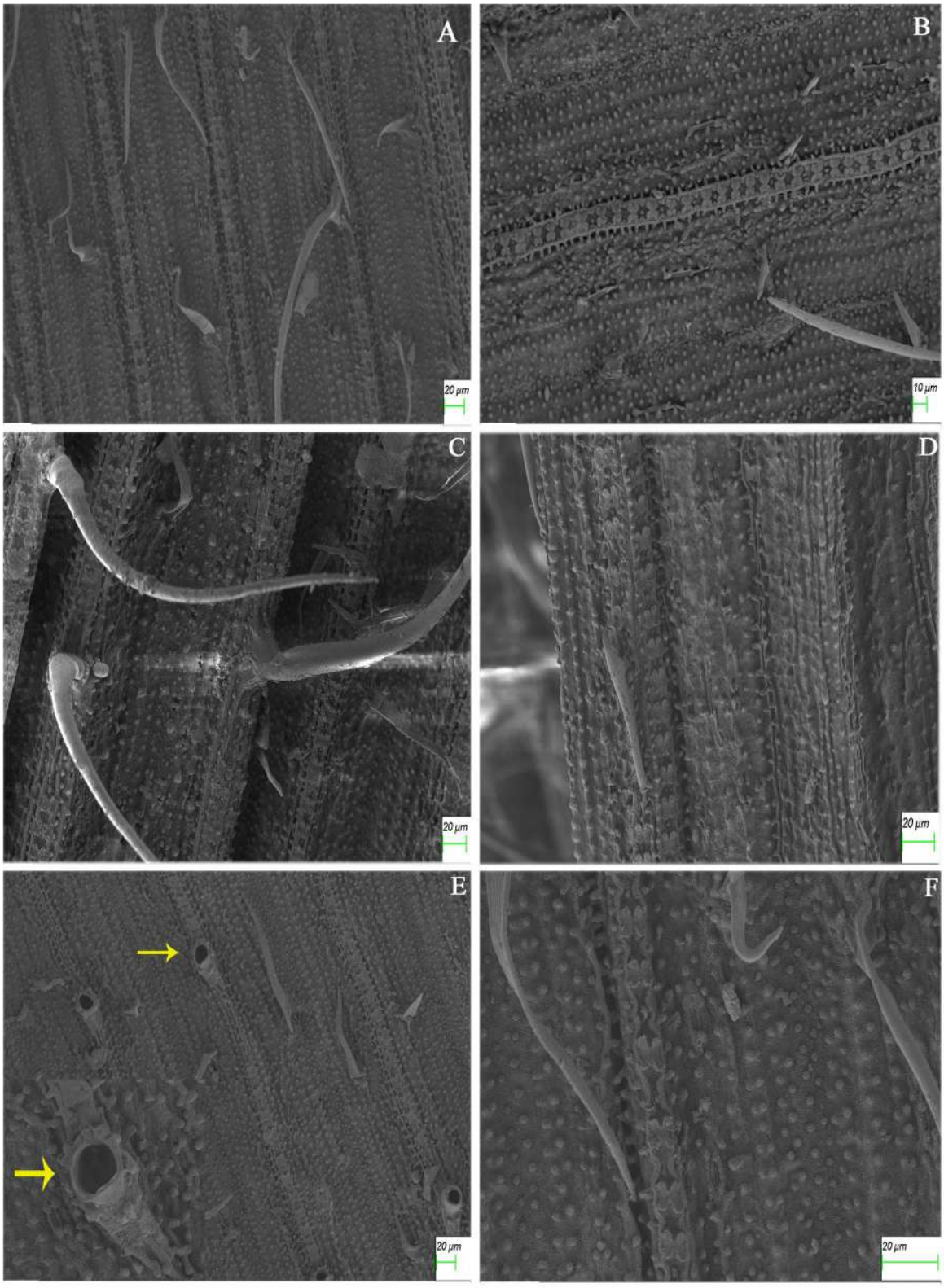

Figure 28: Scanning electron micrographs of adaxial leaf surface of *O. sativa* associated with mycorrhiza and exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd D) AM+Cd, E) non-AM+Zn and F) AM+Zn.

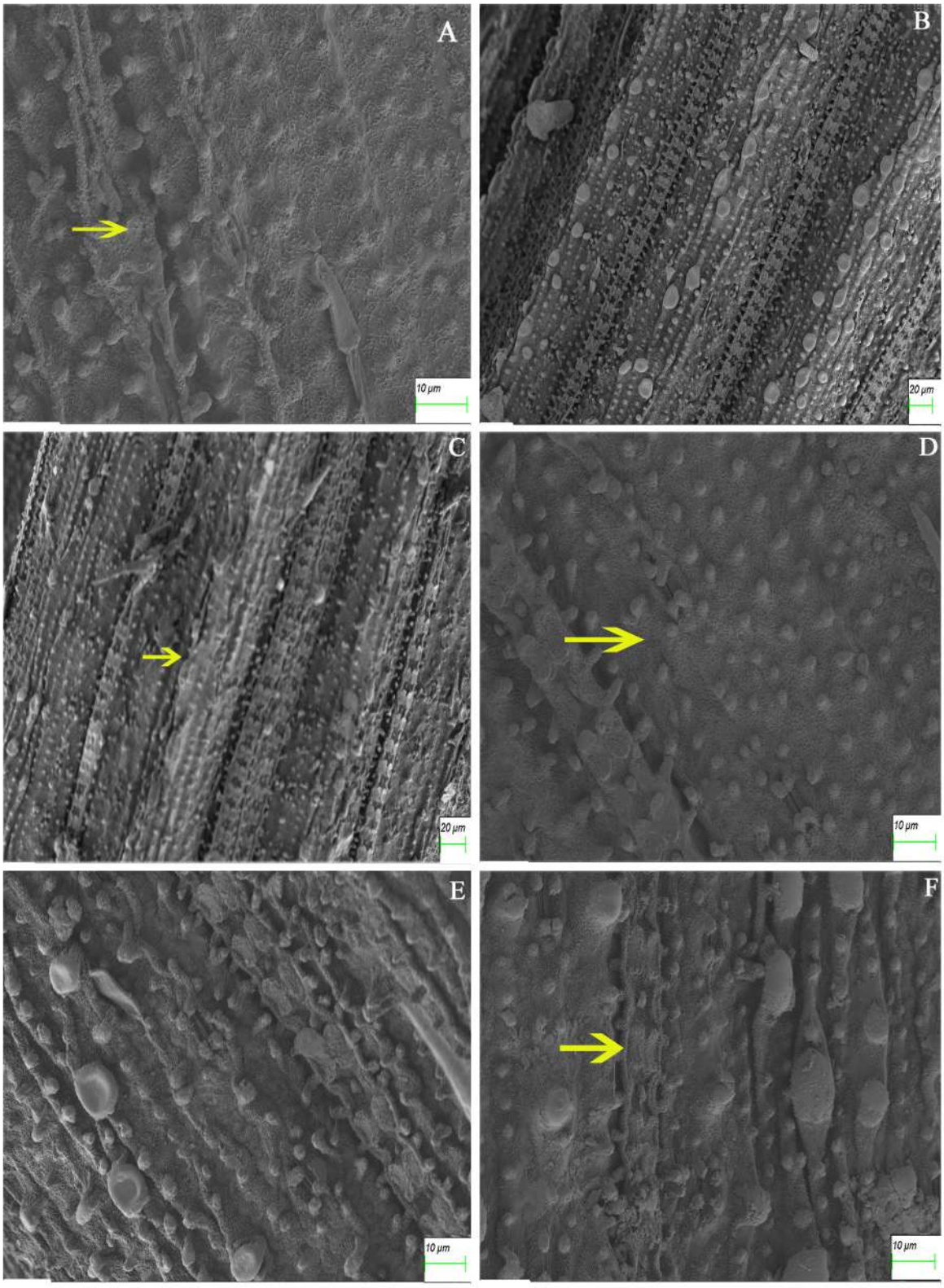

Figure 29: Scanning electron micrographs of abaxial leaf surface of *O. sativa* associated with mycorrhiza exposed to Cd (0.45 g kg^{-1}) and Zn (1. 95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd D) AM+Cd, E) non-AM+Zn and F) AM+Zn.

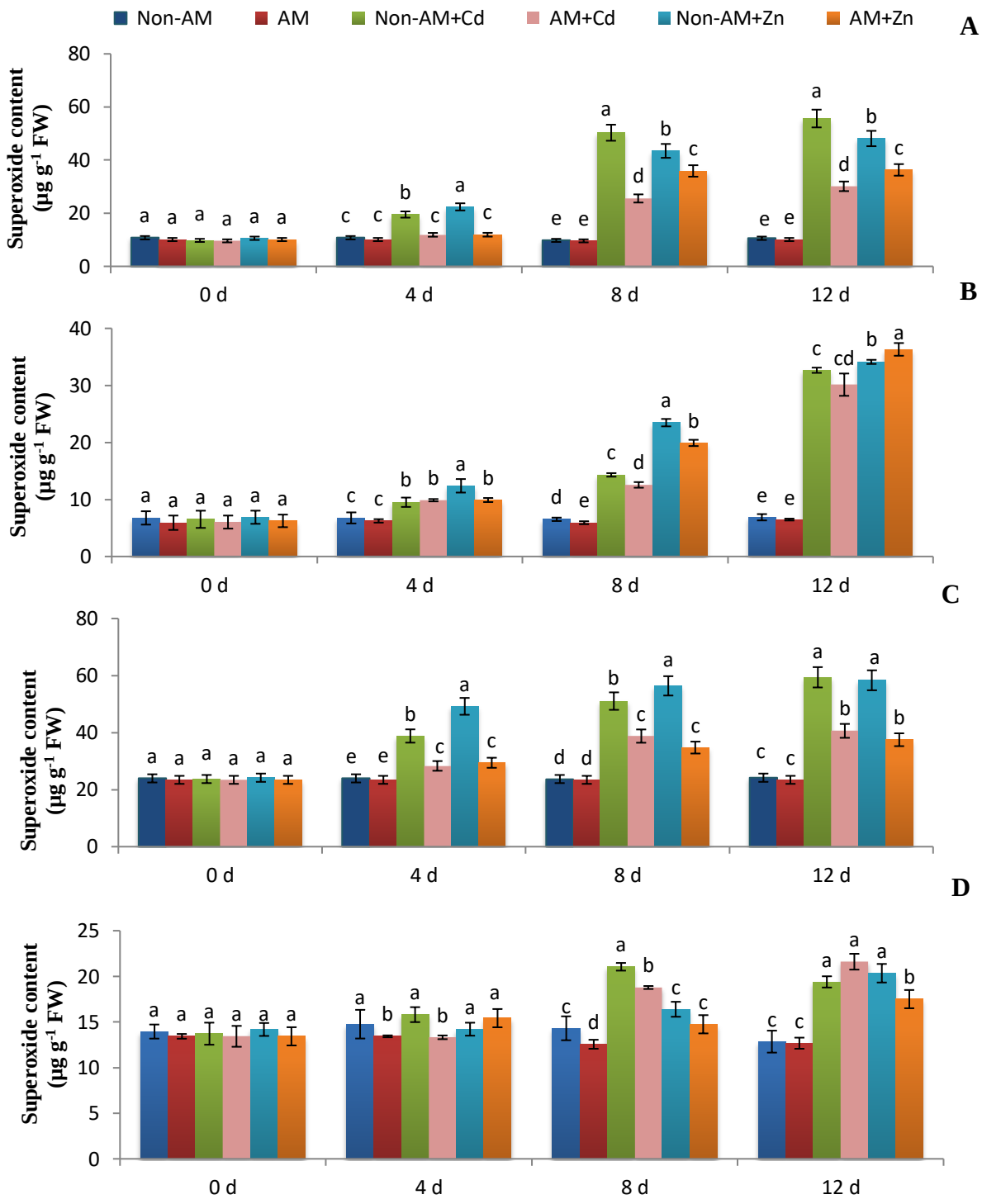

Figure 30: Superoxide content in non-AM and AM plants A) leaves in *Z. mays*, B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

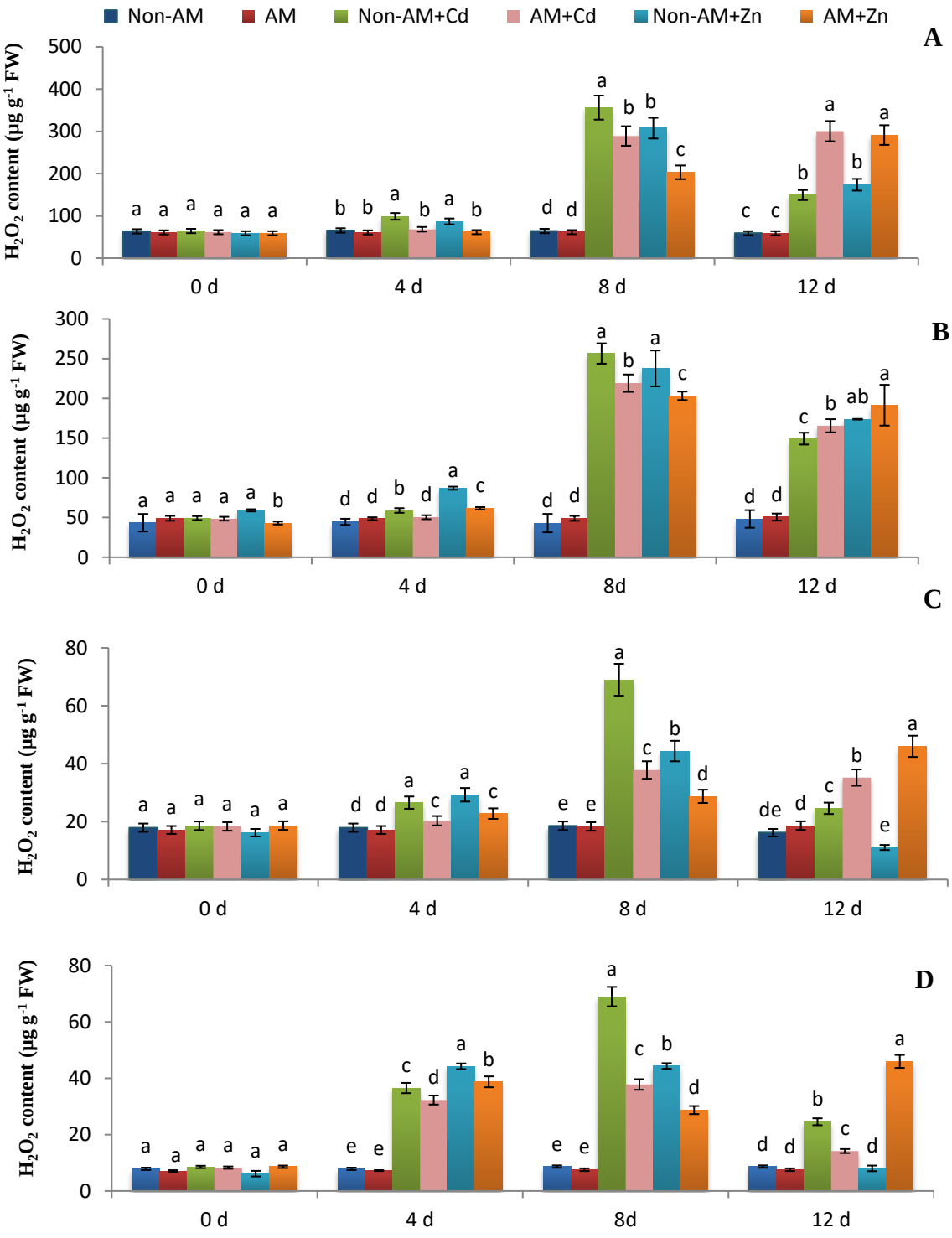

Figure 31: Hydrogen peroxide content in non-AM and AM plants A) leaves of *Z. mays*, B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

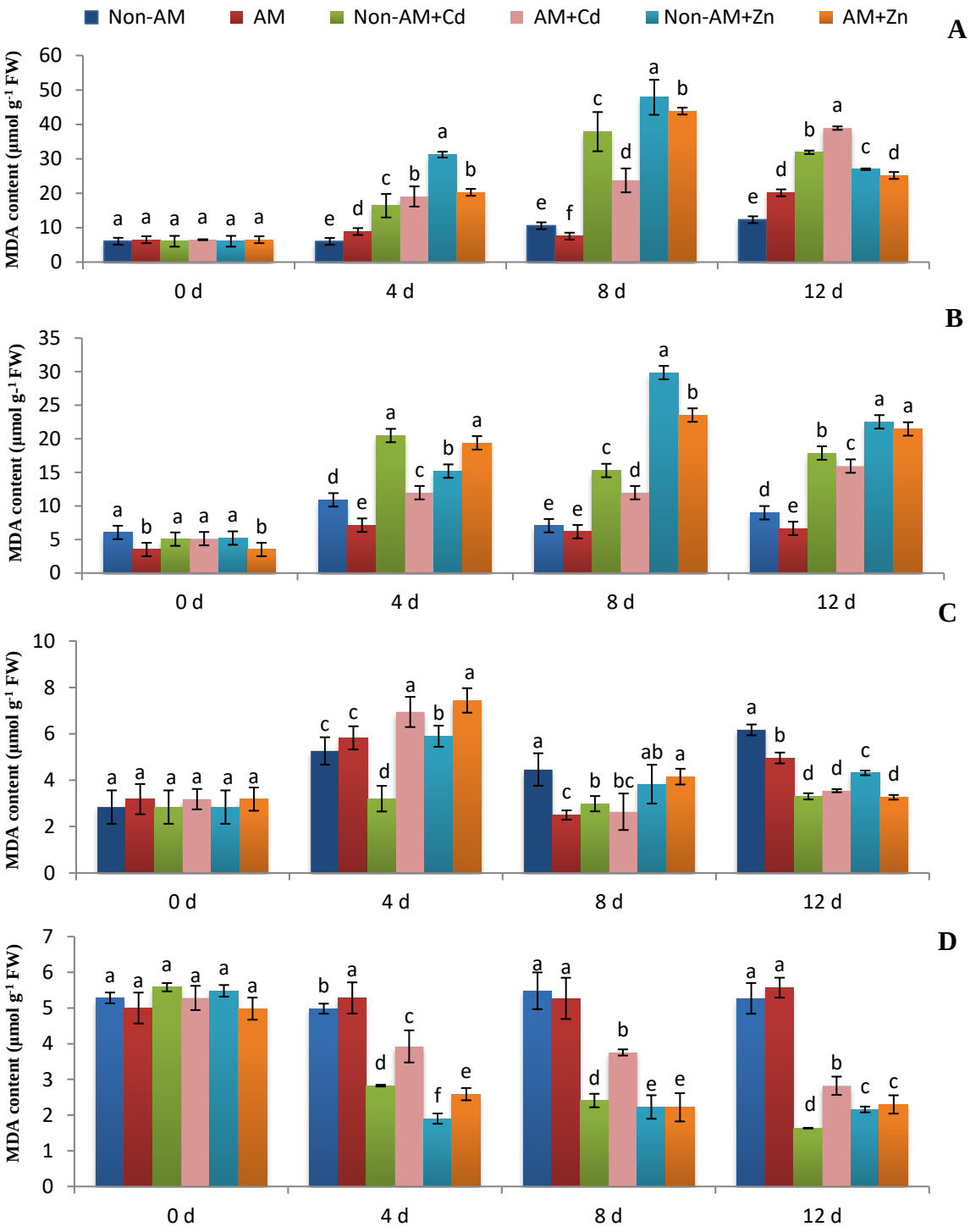

Figure 32: Malondialdehyde content in non-AM and AM plants A) leaves of *Z. mays*, B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

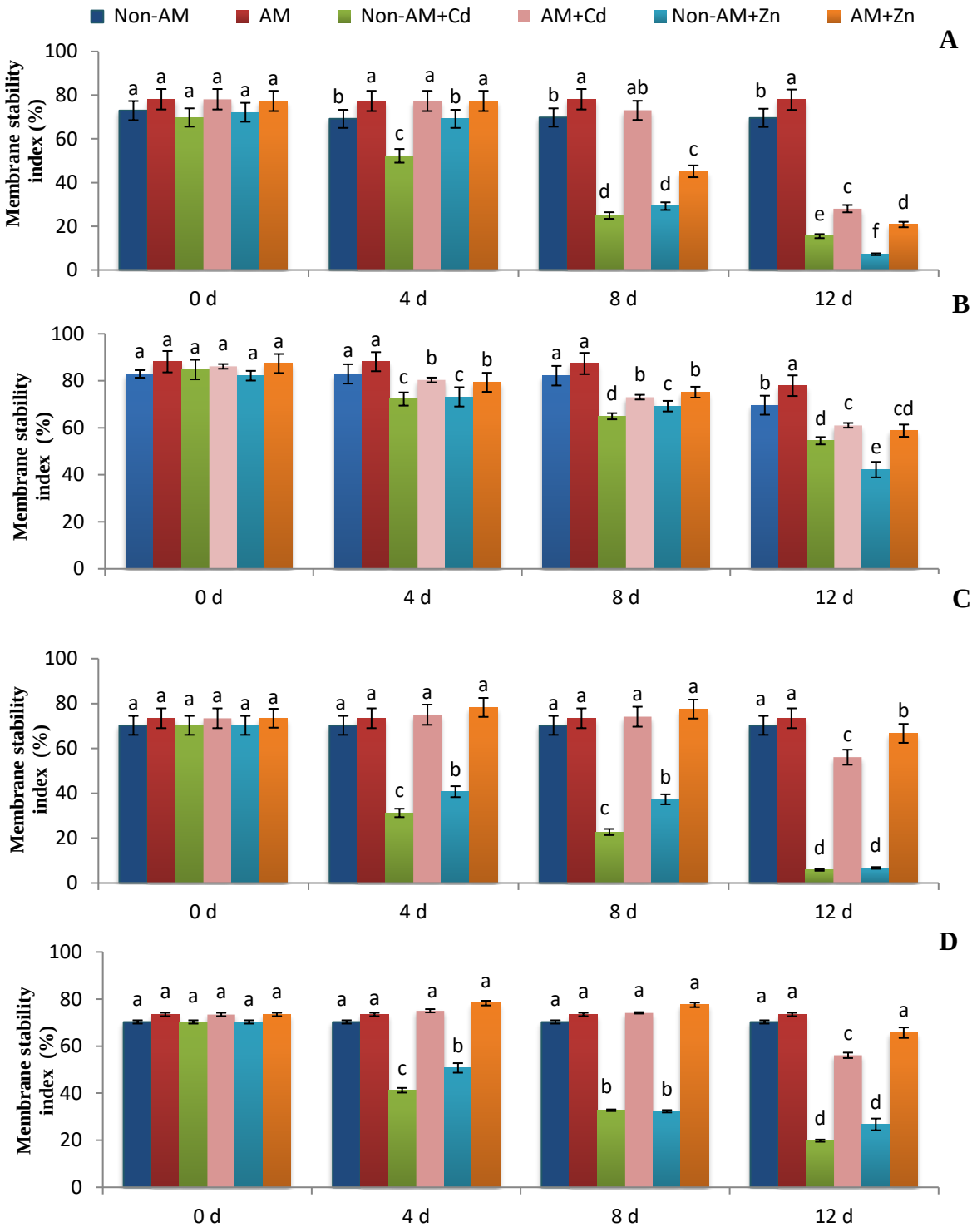

Figure 33: Membrane stability index of non-AM and AM plants A) leaves of *Z. mays*, B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

Table 10: Pearson's correlation coefficients between various parameters related to photosynthesis, ROS, and MDA content in the leaves of non-AM plants of *Z. mays* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses.

	MDA	$O_2^{\cdot-}$	H_2O_2	Total chlorophyll	Fv/FM	tFm	Area	PI_{ABS}	ABS/RC	DIo/RC	TRo/RC	ETo/RC	DF_{ABS}	N	PSII activity	PSI activity
MDA	1	.913**	.915**	-.997**	-.612	.258	-.611	-.859**	.999**	.713*	-.037	-.596	-.669*	-.430	-.841**	-.775*
$O_2^{\cdot-}$		1	1.00**	-.877**	-.880**	.628	-.881**	-.993**	.895**	.937**	-.442	-.872**	-.914**	-.761*	-.989**	-.965**
H_2O_2			1	-.880**	-.878**	.624	-.878**	-.993**	.897**	.935**	-.437	-.869**	-.912**	-.758*	-.988**	-.964**
Total chlorophyll				1	.547	-.180	.545	.815**	-.999**	-.655	-.042	.529	.608	.357	.796*	.722*
Fv/FM					1	-.917**	.998**	.929**	-.579	-.989**	.810**	.997**	.995**	.975**	.940**	.972**
tFm						1	-.919**	-.714*	.218	.858**	-.972**	-.926**	-.887**	-.980**	-.735*	-.806**
Area							1	.930**	-.577	-.991**	.814**	1.000**	.997**	.978**	.942**	.974**
PI_{ABS}								1	-.837**	-.971**	.543	.923**	.955**	.832**	.999**	.989**
ABS/RC									1	.683*	.004	-.561	-.638	-.392	-.818**	-.748*
DIo/RC										1	-.727*	-.988**	-.998**	-.940**	-.979**	-.995**
TRo/RC											1	.825**	.767*	.918**	.571	.660
ETo/RC												1	.995**	.981**	.935**	.969**
DF_{ABS}													1	.959**	.965**	.988**
N														1	.849**	.904**
PSII activity															1	.994**
PSI activity																1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 11: Pearson's correlation coefficients between various parameters related to photosynthesis, ROS, and MDA content in the leaves of AM plants of *Z. mays* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses.

	MDA	$O_2^{\cdot-}$	H_2O_2	Total chlorophyll	Fv/FM	tFm	Area	PI_{ABS}	ABS/RC	DIo/RC	TRo/RC	ETo/RC	DF_{ABS}	N	PSII activity	PSI activity
MDA	1	.943**	.577	-.491	.295	.749*	-.124	-.204	-.112	-.390	-.276	.744*	.312	-.092	-.785*	-.716*
$O_2^{\cdot-}$		1	.801**	-.735*	.432	.915**	-.422	-.164	.186	-.212	.011	.510	.008	-.039	-.937**	-.897**
H_2O_2			1	-.994**	.476	.970**	-.879**	.034	.638	.133	.606	-.095	-.586	.284	-.957**	-.977**
Total chlorophyll				1	-.471	-.939**	.924**	-.059	-.695*	-.197	-.682*	.199	.668*	-.325	.921**	.953**
Fv/FM					1	.473	-.406	.003	.325	.497	.169	-.017	-.257	.117	-.466	-.508
tFm						1	-.737*	-.030	.472	-.024	.406	.148	-.373	.174	-.998**	-.992**
Area							1	-.174	-.840**	-.398	-.899**	.559	.902**	-.446	.703*	.769*
PI_{ABS}								1	.365	-.214	.321	-.316	-.270	.548	.067	.102
ABS/RC									1	.476	.806**	-.648	-.851**	.345	-.436	-.497
DIo/RC										1	.447	-.591	-.551	.168	.037	-.070
TRo/RC											1	-.824**	-.979**	.565	-.356	-.435
ETo/RC												1	.862**	-.436	-.198	-.097
DF_{ABS}													1	-.500	.326	.419
N														1	-.153	-.174
PSII activity															1	.991**
PSI activity																1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 12: Pearson's correlation coefficients between various parameters related to photosynthesis, ROS, and MDA content in the leaves of non-AM plants of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses.

	MDA	$O_2^{\cdot-}$	H_2O_2	Total chlorophyll	Fv/FM	tFm	Area	PI_{ABS}	ABS/RC	DIo/RC	TRo/RC	ETo/RC	DF_{ABS}	N	PSII activity	PSI activity
MDA	1	.977**	.733*	-.562	.095	.425	-.671*	-.782*	.217	.067	.323	-.452	-.349	-.854**	-.693*	-.868**
$O_2^{\cdot-}$		1	.801**	-.640	.061	.514	-.743*	-.757*	.302	.154	.415	-.537	-.436	-.907**	-.762*	-.897**
H_2O_2			1	-.972**	-.146	.924**	-.996**	-.262	.810**	.714*	.876**	-.935**	-.888**	-.978**	-.982**	-.952**
Total chlorophyll				1	.217	-.987**	.989**	.041	-.923**	-.86**	-.963**	.992**	.970**	.903**	.967**	.871**
Fv/FM					1	-.228	.184	-.166	-.249	-.288	-.295	.245	.254	.109	.044	-.030
tFm						1	-.954**	.109	.969**	.926**	.992**	-.999**	-.996**	-.825**	-.928**	-.791*
Area							1	.179	-.858**	-.774*	-.916**	.963**	.925**	.955**	.981**	.925**
PI_{ABS}								1	.342	.459	.212	-.081	-.191	.449	.181	.442
ABS/RC									1	.986**	.986**	-.963**	-.986**	-.670*	-.837**	-.639
DIo/RC										1	.960**	-.916**	-.956**	-.553	-.738*	-.519
TRo/RC											1	-.989**	-.998**	-.758*	-.879**	-.720*
ETo/RC												1	.993**	.841**	.935**	.806**
DF_{ABS}													1	.773*	.894**	.738*
N														1	.947**	.976**
PSII activity															1	.929**
PSI activity																1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 13: Pearson's correlation coefficients between various parameters related to photosynthesis, ROS, and MDA content in the leaves of AM plants of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses.

	MDA	$O_2^{\cdot-}$	H_2O_2	Total chlorophyll	Fv/FM	tFm	Area	PI_{ABS}	ABS/RC	DIo/RC	TRo/RC	ETo/RC	DF_{ABS}	N	PSII activity	PSI activity
MDA	1	.943**	0.577	-0.491	0.295	.749*	-0.124	-0.204	-0.112	-0.39	-0.276	.744*	0.312	-0.092	-.785*	-.716*
$O_2^{\cdot-}$		1	.801**	-.735*	0.432	.915**	-0.422	-0.164	0.186	-0.212	0.011	0.51	0.008	-0.039	-.937**	-.897**
H_2O_2			1	-.994**	0.476	.970**	-.879**	0.034	0.638	0.133	0.606	-0.095	-0.586	0.284	-.957**	-.977**
Total chlorophyll				1	-0.471	-.939**	.924**	-0.059	-.695*	-0.197	-.682*	0.199	.668*	-0.325	.921**	.953**
Fv/FM					1	0.473	-0.406	0.003	0.325	0.497	0.169	-0.017	-0.257	0.117	-0.466	-0.508
tFm						1	-.737*	-0.03	0.472	-0.024	0.406	0.148	-0.373	0.174	-.998**	-.992**
Area							1	-0.174	-.840**	-0.398	-.899**	0.559	.902**	-0.446	.703*	.769*
PI_{ABS}								1	0.365	-0.214	0.321	-0.316	-0.27	0.548	0.067	0.102
ABS/RC									1	0.476	.806**	-0.648	-.851**	0.345	-0.436	-0.497
DIo/RC										1	0.447	-0.591	-0.551	0.168	0.037	-0.07
TRo/RC											1	-.824**	-.979**	0.565	-0.356	-0.435
ETo/RC												1	.862**	-0.436	-0.198	-0.097
DF_{ABS}													1	-0.5	0.326	0.419
N														1	-0.153	-0.174
PSII activity															1	.991**
PSI activity																1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

4.4.2.1 Soluble sugar content

Both Cd and Zn stresses augmented the soluble sugar content in the leaves of non-AM and AM plants of *Z. mays* on initial days of exposure to metal stress (Fig. 35A). The increase was 132-167% in Cd and Zn treated non-AM plants and AM plants on the 8 d of exposure. On 12 d, AM plants accumulated more sugar content under Cd and Zn stresses, but in non-AM plants the sugar content was reduced (28-34%) as compared to the control. The augmentation in soluble sugar content was 3 and 4 folds in Cd and Zn treated non-AM plants of *O. sativa*, but it was only 2 and 3 folds in AM plants on the 4 d of exposure. Similar to *Z. mays*, the sugar content in AM plants of *O. sativa* was higher on 12 d of imparting Cd and Zn stresses (Fig. 35A and B).

In *Z. mays*, the sugar content was decreased in roots of non-AM and AM plants on the 12 d of exposure to Cd and Zn stresses (Fig. 35C). The reduction was to an extend of 86-92% in non-AM plants, but was only 35-44% in AM plants under Cd and Zn stresses. Although sugar content was increased in roots of non-AM and AM *O. sativa* plants on the 4 d of exposure to Cd and Zn stresses. In further days of stress, the sugar content was maintained to a level same as that of control in AM plants alone, not in non-AM plants (Fig. 35D).

4.4.2.2 Soluble protein content

Soluble protein content in leaves of *Z. mays* and *O. sativa* increased on exposure to Cd and Zn stresses (Fig. 36A and B). On 4 d of Cd and Zn exposure, there was a significant increase in the protein content in AM plants of *Z. mays* and it was to the extent of 141-182% but the increase was only 71 and 45% in non-AM plants of *Z. mays*. The protein content recorded on 8 d was lesser than that recorded on 4 d in non-AM plants of protein content was

increased to the extent of 111-147% in non-AM plants of *O. sativa* on 4 d of imparting Cd and Zn exposure but the increase was insignificant in Cd treated AM plants. At the same time, the increase was 144% in AM plants exposed to Zn stress. In further days of treatment, the protein content was reduced in *O. sativa*, and the reduction was higher in non-AM plants as compared to AM plants.

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. 36C and D). On 12 d of Cd stress, the protein content was insignificantly reduced in of non-AM plants, at the same time, there was a 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. In the case of *O. sativa*, the reduction was 15 and 23% in Cd and Zn treated non-AM plants respectively, but the reduction was insignificant in AM plants on 12 d of Cd and Zn stresses.

4.4.2.3 Amino acids content

Cadmium and Zn stresses elicited amino acids accumulation in the leaves of non-AM and AM plants of *Z. mays* (Fig. 37A and B). 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 an extent of 58% in Cd treated non-AM plants, but not in the case of AM plants. Similar to that of *Z. mays*, Cd and Zn stresses elicited amino acids accumulation in the leaves of non-AM and AM plants of *O. sativa*. The augmentation was 9 and 7 fold in Cd and Zn treated non-AM plants, but it was only 5 and 4 fold in AM plants on 4 d of exposure.

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 an extent of 64-65%,

but the reduction was only 13 and 26% in Cd and Zn treated AM plants (Fig. 37C). In the case of *O. sativa*, amino acids content was decreased in the roots of non-AM and AM plants on 12 d of exposure to Cd and Zn stresses and it was to the extent of 53 and 31% in Cd and Zn treated plants, but the reduction was only 15 and 8% in Cd and Zn treated AM plants (Fig. 37D).

4.4.2.4 Proline content

Both Cd and Zn stresses induced an increase in the proline content in the leaves of non-AM and AM plants of *Z. mays* (Fig. 38A). 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 content occured on 8 d of Cd and Zn stresses. In *O. sativa*, both Cd and Zn stresses induced an increase in the proline content in the leaves of non-AM and AM plants (Fig. 38B). The enhancement in proline content was up to 9 and 18 fold in non-AM plants under Cd and Zn stresses respectively as compared to the control. But, in the leaves of AM plants, only 2 and 10 fold increment in proline content occured 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, where as the reduction was only 9-12% in AM associated roots (Fig. 38C). Cadmium and Zn stresses reduced the proline content in *O. sativa* and the reduction was 21-23% in Cd and Zn treated non-AM plants, where as the reduction was only 9-11% in AM associated roots (Fig. 38D).

4.4.3 Secondary metabolites

The accumulation of secondary metabolites such as phenolics, anthocyanin, flavonoids, alkaloids and other bioactive compounds

significantly varied in non-AM and AM plants of *Z. mays* and *O. sativa* on exposure to Cd and Zn stresses.

4.4.3.1 Phenolics content

Phenolics content was increased to an extent of 59 and 84% in the leaves of non-AM plants of *Z. mays*, on exposure to Cd and Zn stresses respectively. However, the increase was only 44% in Cd treated AM plants and it was insignificant in AM plants exposed to Zn stress on 4 d (Fig. 39A). However, this trend of phenolics accumulation changed with the extension of days of exposure to stress and the AM plants accumulated more phenolics content on 8 d of exposure to Cd and Zn stresses. In the case of *O. sativa*, phenolics content was increased to an extent of 154 and 101% in the leaves of non-AM plants on exposure to Cd and Zn stresses respectively, and the increase was only up to 125 and 76% in AM plants on 4 d of stress (Fig. 39B). But this trend of phenolics accumulation changed with the progression in the days of exposure and AM plants accumulated higher phenolics content on 12 d of exposure to Cd and Zn stresses (Fig. 39A and B).

In roots, phenolics content was decreased in non-AM and AM plants of *Z. mays* on exposure to Cd and Zn stresses (Fig. 39C). 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. Similarly, in *O. sativa,* phenolics content was decreased in roots of non-AM and AM plants on exposure to Cd and Zn stresses. The reduction was 25 and 12% in non-AM plants, and there was a negligible reduction of this metabolite in AM plants as compared to the control on 8 d of Cd and Zn stresses (Fig. 39D).

4.4.3.2 Flavonoids content

Both Cd and Zn stresses induced an increase in the flavonoids content in the leaves of non-AM and AM plants of *Z. mays* and *O. sativa* (Fig. 40A and B). In *Z. mays*, non-AM plants enhanced the flavonoids content by 15-26% under Cd and Zn stresses as compared to the control. But, in the leaves of AM plants, only 17-19% increment in flavonoids content was observed on 4 d of Cd and Zn stresses. The flavonoids content of non-AM *O. sativa* increased by 26-31% on 4 d of Cd and Zn stresses and the increase was 13-15% in AM plants.

Contrary to that of shoots, the flavonoids content in roots of non-AM and AM plants was reduced on exposure to Cd and Zn stresses (Fig. 40C and D). In *Z. mays*, the reduction in flavonoids content was 62-67% in roots of Cd and Zn treated non-AM plants, whereas the reduction was insignificant in AM associated roots on 12 d of stress. There was 70 and 54% of increase in flavonoids content in roots of non-AM plants of *O. sativa* on 12 d of exposure to Cd and Zn stresses respectively but in AM plants the increase was negligible.

4.4.3.3 Anthocyanin content

Cd and Zn stresses elicited anthocyanin accumulation in the leaves of non-AM and AM plants of *Z. mays* and *O. sativa* (Fig. 41A and B). In *Z. mays*, the increase was 4 and 9 fold in Cd and Zn treated non-AM plants, and it was 1.8 and 10 fold in AM plants on 8 d of exposure. Whereas in *O. sativa*, the increase in anthocyanin content was 66 and 59% in non-AM plants exposed to Cd and Zn stresses respectively and the increase was up to 42-45% in AM plants on 8 d of exposure.

Anthocyanin content was also increased in the roots of *Z. mays*. In non-AM plants the increase was to the extent of 25% under Cd stress,

whereas the increase was 32% in AM plants exposed to Cd stress (Fig. 41C). Zinc toxicity did not induce any significant change in the anthocyanin content of roots of *Z. mays*. In *O. sativa*, anthocyanin content was reduced (25-29%) in the non-AM and AM plants exposed to Cd and Zn stresses on 8 d of exposure (Fig. 41D).

4.4.3.4 Alkaloids content

Cadmium and Zn stresses induced an increase in the alkaloids content in the leaves of non-AM plants of *Z. mays* and it was to an extent of 27 and 42%. In AM plants, the enhancement in alkaloids content under Cd stress was only 13%, and it decreased (27%) on 4 d of Zn stress (Fig. 42A). In *O. sativa*, the increase in alkaloids content was 20% in Cd treated non-AM plants and the increase was insignificant in AM plants exposed to Cd. The increase was also insignificant in non-AM and AM plants on 4 d of Zn stress (Fig. 42B).

Both Cd and Zn stresses reduced the alkaloids content in roots of non-AM and AM plants of *Z. mays* and *O. sativa* (Fig. 42C and D). The reduction in alkaloids content was 24-38% in Cd and Zn treated non-AM plants of *Z. mays*, whereas the reduction was only 8-31% in AM associated roots. In *O. sativa*, the reduction was up to 23-24% in non-AM plants and insignificant in AM plants on 8 d of exposure to Cd and Zn stresses.

4.4.3.5 Bioactive compounds

The phytochemical composition of leaves and roots of non-AM and AM plants of *Z. mays* and *O. sativa* was significantly altered under Cd and Zn stresses (Table 14, 15, 16, 17, and 18).

In the case of *Z. mays* leaves, neophytadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, (E)-phytol, methyl palmitate, octadecadienoic acid, squalene, hahnfett, methyl linolenate and phytol were the different bioactive compounds

detected commonly in the leaves of non-AM and AM plants. 1,2-benzenedicarboxylic acid and cholesta-4,6-dien-3-ol, benzoate, (3beta)- were specific to the leaves of mycorrhizal plants. Cadmium induced the accumulation of gamma-sitosterol, 3beta-acetoxystigmasta-4,6,22-triene and cholesta-4,6-dien-3-ol, benzoate, (3beta) in non-mycorrhizal plants. At the same time, Cd stress elicited the production of beta-linalool and gamma-sitosterol in mycorrhizal plants (Table 14).

Exposure to Cd stress also leads to a drastic reduction in the content of neophytadiene in mycorrhizal and non-mycorrhizal plants. As compared to the control plants, Zn stress induced the accumulation of octadecadien-1-ol and cholesta-4,6-dien-3-ol, benzoate in AM and non-AM plants. Moreover, exposure to Zn stress caused a modification in the ratio between phytol and (E)-phytol. Two forms of phytol with two different retention time (RT) were detected in the methanolic extract of *Z. mays* leaves, *viz.* (E)-phytol (RT-27.387) and phytol (RT-31.849). In the present study, Cd stress altered the ratio between phytol and (E)-phytol in both non-AM and AM plants. The alteration in the ratio between phytol and (E)-phytol was significant and it was metal dependent. The ratio was 2.3 and 1.9 in non-AM and AM plants, but under Cd toxicity it was increased to 4 in both non-AM and AM plants. When the non-AM and AM plants was exposed to Zn toxicity, the ratio between phytol and (E)-phytol was reduced to 0.8 (Table 16).

In *O. sativa*, neophytadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, (E)-phytol, phytol, 1,2-benzenedicarboxylic acid di-iso-octyl ester and ethyl iso-allocholate were the different bioactive compounds detected common in the leaves of non-AM and AM plants (Table 15).

Cadmium stress induced the accumulation of cholesta-4,6-dien-3-ol, benzoate in the leaves of non-AM and AM plants and (3beta)- and dihydroergosterol was formed specifically in non-AM plants. Cadmium stress

also lead to a drastic reduction in the content of neophytadiene in mycorrhizal and non-mycorrhizal plants. Another compound, (E)-phytol was not detected in non-AM plants, but it was detected only in mycorrhizal plants on exposure to Cd stress (Table 15).

2-pentadecanone, 6,10,14-trimethyl-, and 2-hexadecen-1-ol, 3,7,11,15-tetramethyl-hexadecen-1-ol were the compounds commonly accumulated in the leaves of non-AM and AM plants of *O. sativa* under Zn toxicity. Whereas 3beta-acetoxystigmasta-4,6,22-triene, cyclopropaneoctanoic acid was characteristic of non-AM plants under Zn toxicity. Zinc also caused a reduction of neophytadiene in both non-AM and AM plants. In non-AM plants exposed to Zn toxicity, the content of phytol was drastically reduced to 3.68%, but it was 21.83% in AM plants (Table 15).

Mycorrhizae and heavy metal induced alterations in the chemical constituents in the roots of *Z. mays* and *O. sativa* was also very much pronounced (Table 17 and 18).

1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, 1,2-benzenedicarboxylic acid, gamma-sitosterol, eicosane, tetracosanoic acid methyl ester, squalene, nonadecane, n-nonadecanol-1, methyl stearate, methyl palmitate, methyl isopimarate, methyl 9,12-octadecadienoate, glycerol beta-palmitate, longifolenbromid-I, diethylene glycol dibenzoate, cholesta-4,6-dien-3-ol, (3beta.)-, 3beta-acetoxystigmasta-4,6,22-triene, and 10-octadecenoic acid, methyl ester were the compounds detected in the roots of non-AM *Z. mays* plants. Different from this, 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, 10-octadecenoic acid methyl ester, 4,22-stigmastadiene-3-one, 9,19-cyclolanost-24-en-3-ol, (3beta)-, cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate, stigmasta-5,22-dien-3-ol, (3beta,22e)-, oleic acid, propyl ester, octadecane, methyl palmitate, methyl 9,12-octadecadienoate, hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)

ethyl ester, heptadecane were additionally detected in mycorrhizal roots of *Z. mays* (Table 17).

Cadmium and Zn stresses induced significant alterations in the phytochemistry of root. 16-beta-hydroxydigitoxigenin, elixene, linoleic acid, methyl ester, and methyl melissate were the Cd stress induced compounds detected in the *Z. mays* roots. Furthermore, mycorrhization induced the production of retinol, acetate, pentacosane, oxalic acid, heptadecyl hexyl ester, oleic acid, methyl ester, and l-norvaline, n-(2-methoxyethoxycarbonyl)-, hexadecyl ester in *Z. mays* roots on exposure to Cd stress. Zinc dependent phytochemical changes were also observed in roots, which was evidenced by the detection of new biomolecules in the roots as result of Zn stress. 2-pentadecanone, 6,10,14-trimethyl-, cycloartenol, eicosanoic acid, methyl ester, longifolenbromid-I, and solanesol were the Zn induced compounds observed in non-AM plants. Different from the non-AM plants, mycorrhizal association increased the production of 4,22-stigmastadiene-3-one, eicosane, 9,19-cyclolanost-24-en-3-ol, (3beta)-heptadecane, hexahydrofarnesyl acetone, and 7-hexadecanol in the *Z. mays* roots exposed to Zn stress (Table 17).

In the roots of *O. sativa*, methyl palmitate, octadecenoic acid, methyl ester, methyl stearate, 1,2-benzenedicarboxylic acid were commonly observed in non-AM and AM plants. Mycorrhization could induce the additional production of 3-beta-acetoxystigmasta-4, 6, 22-triene, cholesta-4,6-dien-3-ol, benzoate, eicosane, heptadecane, methyl lignocerate and 2-pentadecanone, 6,10,14-trimethyl (Table 18).

Under Cd stress, stigmasterol was one of the most important chemical constituent observed in non-AM roots and eicosane, ethyl iso-allocholate, N-hentriacontanol-1, and oleic acid, propyl ester were the other compounds detected in non-AM plants. Phytol, tributyl acetylcitrate, tetracosane, 3-ethyl-,1-heneicosanol, 2-methyloctacosane, linoleic acid and L-norvaline were

induced in the mycorrhizal roots. Phytol, tributyl acetylcitrate, 1,2-benzenedicarboxylic acid and 3beta-acetoxystigmasta-4,6,22-triene were the different compounds elicited by Zn stress in roots of non-AM and AM plants. Besides these, retinol and hexahydrofarnesyl acetone were observed in roots of non-AM plants on exposure to Zn stress (Table 18).

4.4.4 Antioxidants activity

4.4.4.1 Non enzymatic antioxidants

4.4.4.1.1 Ascorbate content

Ascorbate content of *Z. mays* and *O. sativa* was increased in the leaves on exposure to Cd and Zn stresses (Fig. 43A and B). In non-AM plants of *Z. mays* the increase in ascorbate content was up to 176-193% on 8 d of Cd and Zn stresses, and the increase was up to 159 and 277% in AM plants exposed to Cd and Zn stresses respectively. Further, on 12 d of metal exposure the ascorbate content was reduced and the increase was only 25-33% in non-AM plants, whereas the ascorbate content increased to an extent of 64-97% in AM plants. In non-AM plants of *O. sativa*, the increase in ascorbate content was 32 and 70% under Cd and Zn stresses respectively, whereas in AM plants the increase was up to 20 and 73% on 8 d of imparting Cd and Zn stresses.

In the roots of *Z. mays,* the ascorbate content was reduced to the extent of 88 and 71% in non-AM plants under Cd and Zn stresses respectively, but the reduction was only 42% in AM plants exposed to 12 d of Cd stress. In Zn treated roots of AM plants of *Z. mays*, 54% of increase was observed in ascorbate content as compared to the control (Fig. 43C). When *O. sativa* was exposed to Cd and Zn stresses, the increase in ascorbate content was up to 20 and 39% in Cd treated non-AM and AM plants respectively. But a reduction in ascorbate was to the extent of 34 and 27% in non-AM and AM plants on 12 of Zn stress (Fig. 43D).

4.4.4.1.2 Glutathione content

Significant increase of the glutathione content was observed in the leaves of *Z. mays* and *O. sativa* on exposure to Cd and Zn stresses (Fig. 44A and B). The increase in glutathione content in non-AM plants of *Z. mays* was 58-62% on 8 d of imparting Cd and Zn stresses. But in AM plants the increase was only to the extent of 39% under Cd and Zn stresses. In the case of *O. sativa* leaves, the increase in glutathione content was up to 115 and 285% in Cd and Zn treated non-AM plants, but it was only 57 and 239% in AM plants. However, on 12 d of imparting Cd and Zn stresses, AM plants had more glutathione content as compared to non-AM plants exposed to 8 d of Cd and Zn stresses.

In roots of *Z. mays*, the glutathione content was increased slightly (15-18%) in non-AM plants on imparting 8 d of Cd and Zn stresses, but the increase was up to 125 and 207% in AM plants. Further, on 12 d of stress, there was a reduction (43-46%) in the glutathione content in non-AM plants but there was an increased level of glutathione content in the roots of AM plants (Fig. 44C). Similar trend was observed in the glutathione content of *O. sativa* roots. On 8 d of imparting Cd and Zn stresses the increase of glutathione content in non-AM plants was 43-46% in non-AM plants and the increase was up to 126% in Cd treated AM plants. The glutathione content of Zn treated AM plants increased slightly as compared to the control. There was a reduction (18-22%) in glutathione content in non-AM plants, but an increase in the level of glutathione content in AM plants as compared to the control on 12 d of imparting Cd and Zn stresses (Fig. 44D).

4.4.4.2 Enzymatic antioxidants

4.4.4.2.1 Superoxide dismutase (SOD, EC 1.15.1.1)

The activity of SOD was increased in the leaves of both *Z. mays* and *O. sativa* on exposure to Cd and Zn stresses (Fig. 45A and B). There was an increase in the activity of SOD in non-AM plants (145-175%) of *Z. mays* on 8 d of imparting Cd and Zn stresses. However, in AM plants, the increase was only up to 54-77% under Cd and Zn stresses. When non-AM plants of *O. sativa* were exposed to Cd and Zn stresses, the increase in activity of SOD was up to 212 and 119% respectively. However, the increase in SOD activity in AM plants was only 65 and 80% on 8 d of imparting Cd and Zn stresses.

In the case of roots, reduction in the activity of SOD (21%) was recorded in non-AM plants of *Z. mays* subjected to Cd stress but it was insignificant in Zn treated plants. However, in AM plants, the activity of SOD was increased and it was to the extent of 40-44% on 8 d of imparting stress (Fig. 45C). In *O. sativa* roots, an increase in the SOD activity was observed in non-AM plants (36-64%). In AM plants, the increase was up to 71 and 44% on 8 d of Cd and Zn stresses respectively (Fig. 45D).

4.4.4.2.2 Catalase (CAT, EC 1.11.1.6)

Metal induced increase in the catalase activity was prominent in the leaves of *Z. mays* and *O. sativa* (Fig. 46A and B). The enhancement of the catalase activity was up to 256 and 158% in non-AM plants of *Z. mays* exposed to 8 d of Cd and Zn stresses and it was only 36 and 90% in Cd and Zn treated AM plants. In the case of *O. sativa,* the increase in the catalase activity was 246 and 233% in non-AM plants, but it was only up to 113 and 198% in AM plants exposed to 8 d of Cd and Zn stresses.

In roots of *Z. mays*, the activity of catalase was increased to the extent of 19-22% in non-AM plants and the increase was up to 42-66% in AM plants exposed to 4 d of Cd and Zn stresses. Further, on 12 d of exposure to Cd and Zn stresses, there was 59-67% reduction in catalase activity of non-AM plants, whereas the reduction was only 25-28% in AM plants (Fig. 46C). In the roots of non-AM of *O. sativa* exposed to Cd and Zn stresses, the reduction in the activity of catalase was up to 33-43%, whereas the reduction rate was only 18% in AM plants under Zn stress. The catalase activity increased by 50% in AM plants exposed to 12 d of Cd stress (Fig. 46D).

4.4.4.2.3 Ascorbate peroxidase (APX, EC 1.11.1.11)

The activity of APX was increased in the leaves of *Z. mays* and *O. sativa* on exposure to Cd and Zn stresses (Fig. 47A and B). The increase in the activity of APX in non-AM *Z. mays* was 309 and 127% on 8 d of imparting Cd and Zn stresses respectively. However, in AM plants, the increase was only up to 94-79% under Cd and Zn stresses. When non-AM plants of *O. sativa* was exposed to Cd and Zn stresses, the increase in APX activity was 204 and 119%. However, there was only 75-80% increase of APX activity in the leaves of AM plants on 8 d of imparting Cd and Zn stresses.

In the case of roots, the activity of APX reduced in non-AM *Z. mays* plants under Cd stress (12%), but it was increased in Zn treated plants up to 21% (Fig. 47C). The activity of APX increased to the extent of 60-65% in AM plants on 8 d of stress. In *O. sativa*, a negligible increase in the APX activity was observed in the roots of non-AM plants (2-21%) exposed to Cd and Zn stresses. Whereas in AM plants, the increase was up to 65 and 80% on 8 d of Cd and Zn stresses respectively (Fig. 47D).

4.4.4.2.4 Guaiacol peroxidase (GPOX, EC 1.11.1.7)

Cadmium and Zn induced enhancement in the activity of GPOX was prominently observed in leaves of *Z. mays* and *O. sativa* (Fig. 48A and B). The increase in the GPOX activity of *Z. mays* was 155 and 100% in non-AM plants exposed to Cd and Zn stresses, and the increase in AM plants was up to 39 and 66% on 8 d of Cd and Zn stresses respectively. In non-AM plants of *O. sativa,* the increase in GPOX activity was up to 298 and 98% under the influence of Cd and Zn stresses respectively, whereas the increase was only up to 119-133% in the leaves of AM plants exposed to 8 d of Cd and Zn stresses.

In roots of non-AM plants of *Z. mays*, the GPOX activity was reduced (16%) under Cd stress and increased (43%) under Zn stress. In AM plants, GPOX activity increased up to 8 and 54% on 8 d of exposure to Cd and Zn stresses (Fig. 48C). In non-AM plants of *O. sativa,* the increase was up to 112 and 64% under the influence of Cd and Zn stresses respectively. In AM plants, the increase was up to 57 and 26% on 8 d of exposure to Cd and Zn stresses (Fig. 48D).

4.4.5 Osmolality

Both Cd and Zn stresses caused an increase in the osmolality of cell sap in the leaves of both non-AM and AM plants of *Z. mays* and *O. sativa* (Fig. 49A and B). In *Z. mays*, 8-9 fold increase in osmolality was observed in non-AM plants on exposure to Cd and Zn stresses, but it was only 3 fold in AM plants as compared to the control on 8 d of the Cd and Zn exposure. On 12 d, the enhancement in osmolality in non-AM plants was up to 3-4 fold, whereas in AM plants the increase was 6-7 fold. The increase in the osmolality was 3-3.6 fold in Cd and Zn treated non-AM plants of *O. sativa*, but it was only 2-2.5 fold in AM plants on 8 d of the stresses.

Osmolality was reduced in roots of non-AM and AM plants of *Z. mays* and *O. sativa* on exposure to Cd and Zn stresses (Fig. 49C and D). In the case of *Z. mays*, the reduction was 37-42% in non-AM plants and there was negligible increase in AM plants on 12 d of exposure to Cd and Zn stresses. The reduction in osmolality was 51 and 19% in Cd and Zn treated non-AM plants of *O. sativa*, but negligible reduction was recorded in AM plants on 12 d of exposure to Cd and Zn stresses.

4.4.6 Characterization of lignin using FTIR

The FTIR spectra of the lignin from the leaves and roots of *Z. mays* and *O. sativa* plants were recorded in the range of 4000-400 cm^{-1} (Fig. 50, 51, 52 and 53).

The spectroscopic patterns of leaf lignin samples were similar in *Z. mays* and *O. sativa* plants exposed to Cd and Zn stresses (Fig. 50 and 52). 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 observed in the case of lignin in the leaves of non mycorrhizal and mycorrhizal plants exposed to Cd stress. However, compared to all other leaf samples, in Zn treated non-AM and AM plants of *Z. mays*, the peaks at 2911 and 2846 cm^{-1} were absent. At the same time, the peaks at 2911 and 2846 cm^{-1} in the lignin of the Zn treated *O. sativa* did not show any significant variations compared to the non treated plants.

In roots also the basic spectroscopic patterns of lignin was almost same in all the samples, but metal treated plants had some differences in the pattern (Fig. 51 and 53). Peaks at 3403, 2911, 2846, 1716, 1624, 1466, 1395, 1322, 1285, 1233, 1178, 1067, 887, 849, 616, 575 and 458 cm^{-1} were observed in the root lignin samples obtained from *Z. mays* and *O. sativa*. Three peaks at 1285, 1233, 1178 cm^{-1} were prominent in the roots of *Z. mays* plants subjected

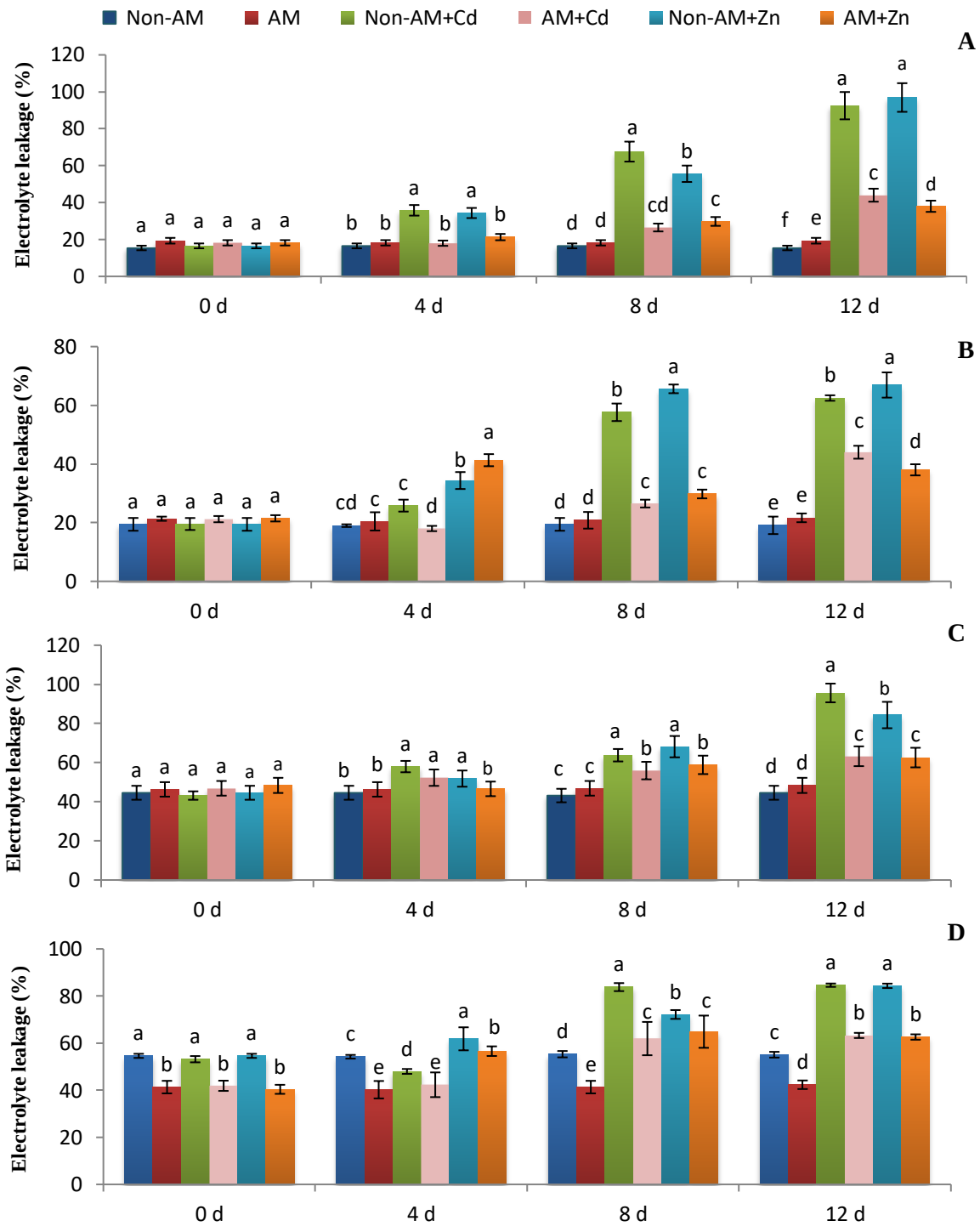

Figure 34: Electrolytes leakage in non-AM and AM plants A) leaves of *Z. mays*, B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

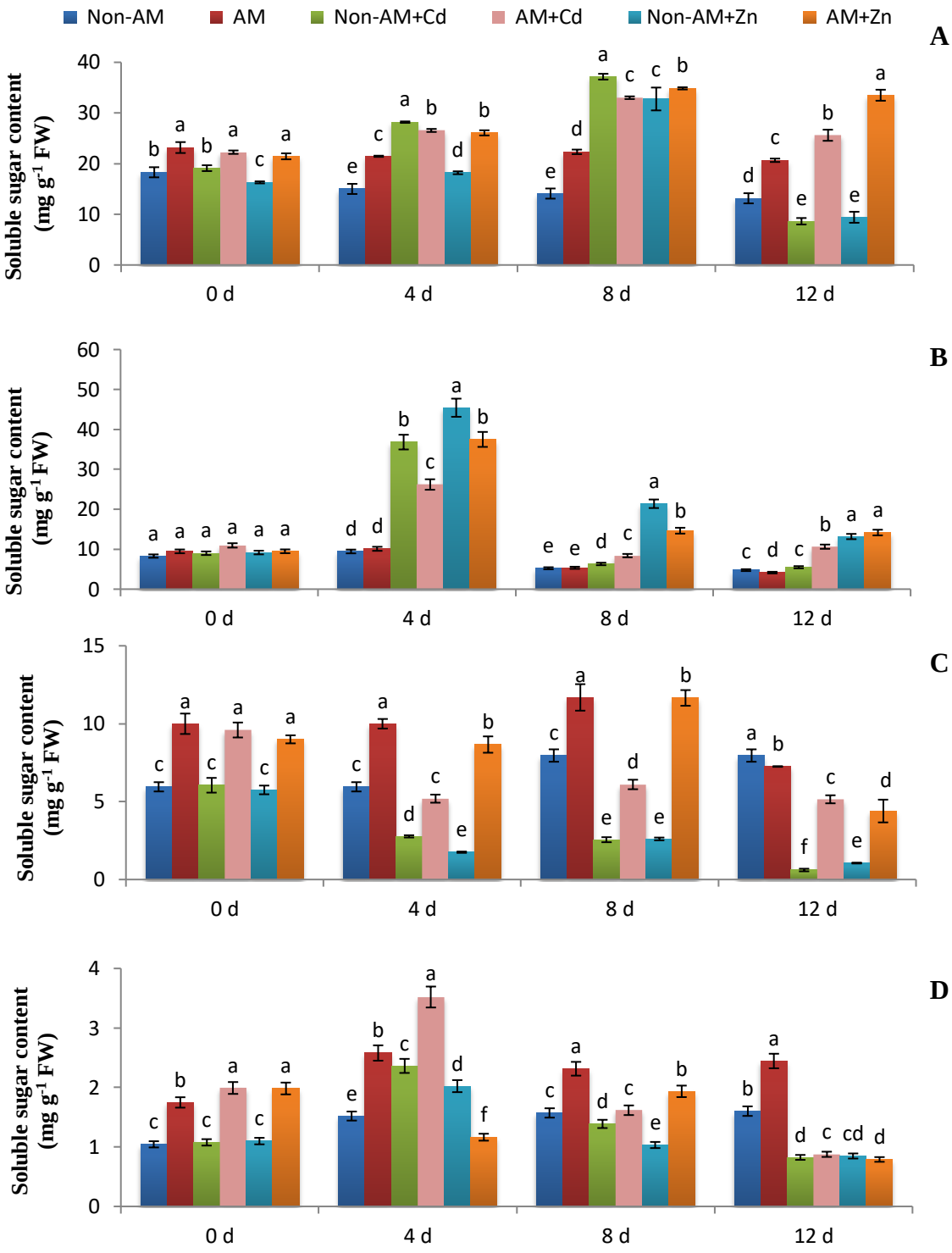

Figure 35: Total soluble sugar content in non-AM and AM plants A) leaves of *Z. mays*, B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

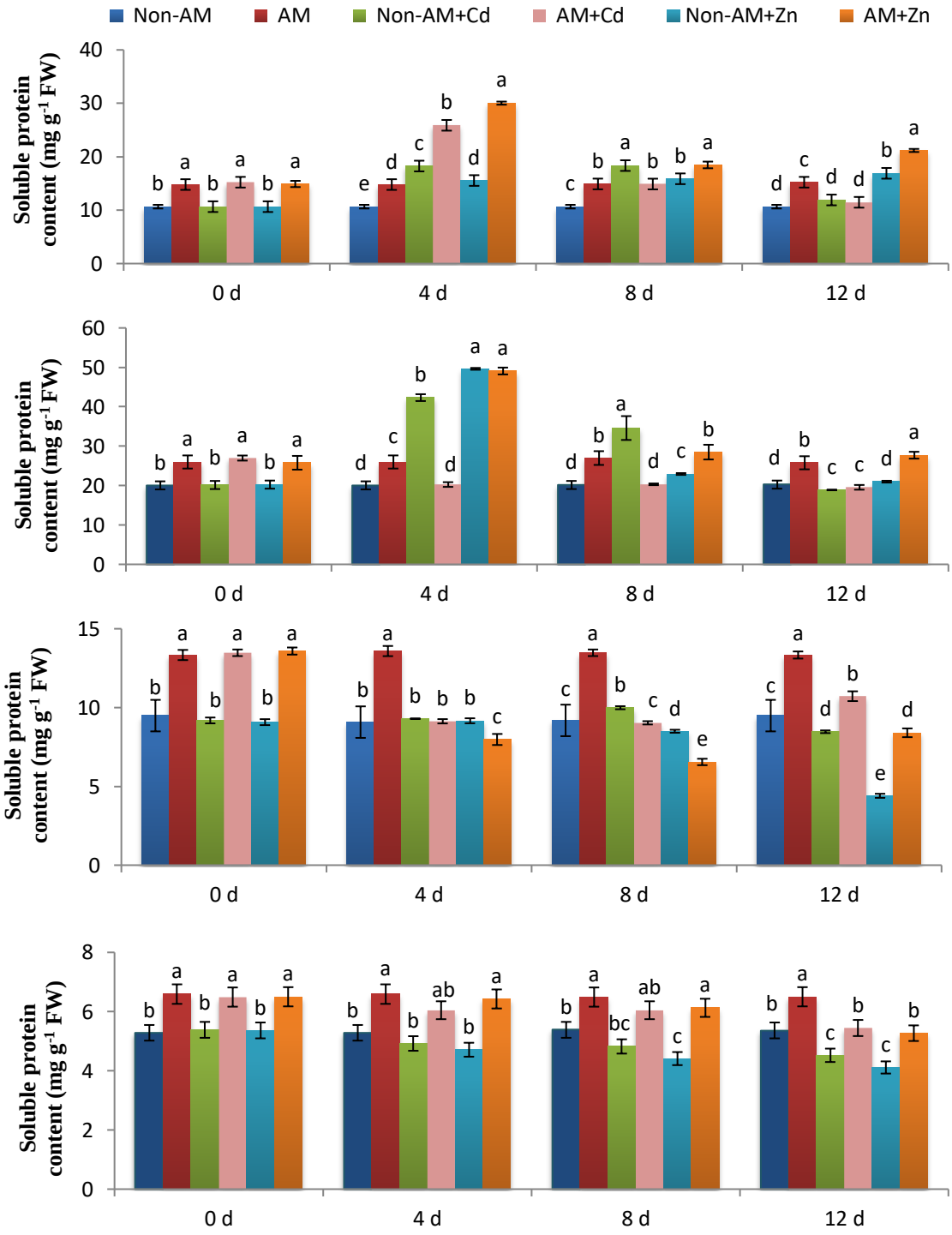

Figure 36: Total soluble protein content in non-AM and AM plants A) leaves of *Z. mays*, B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

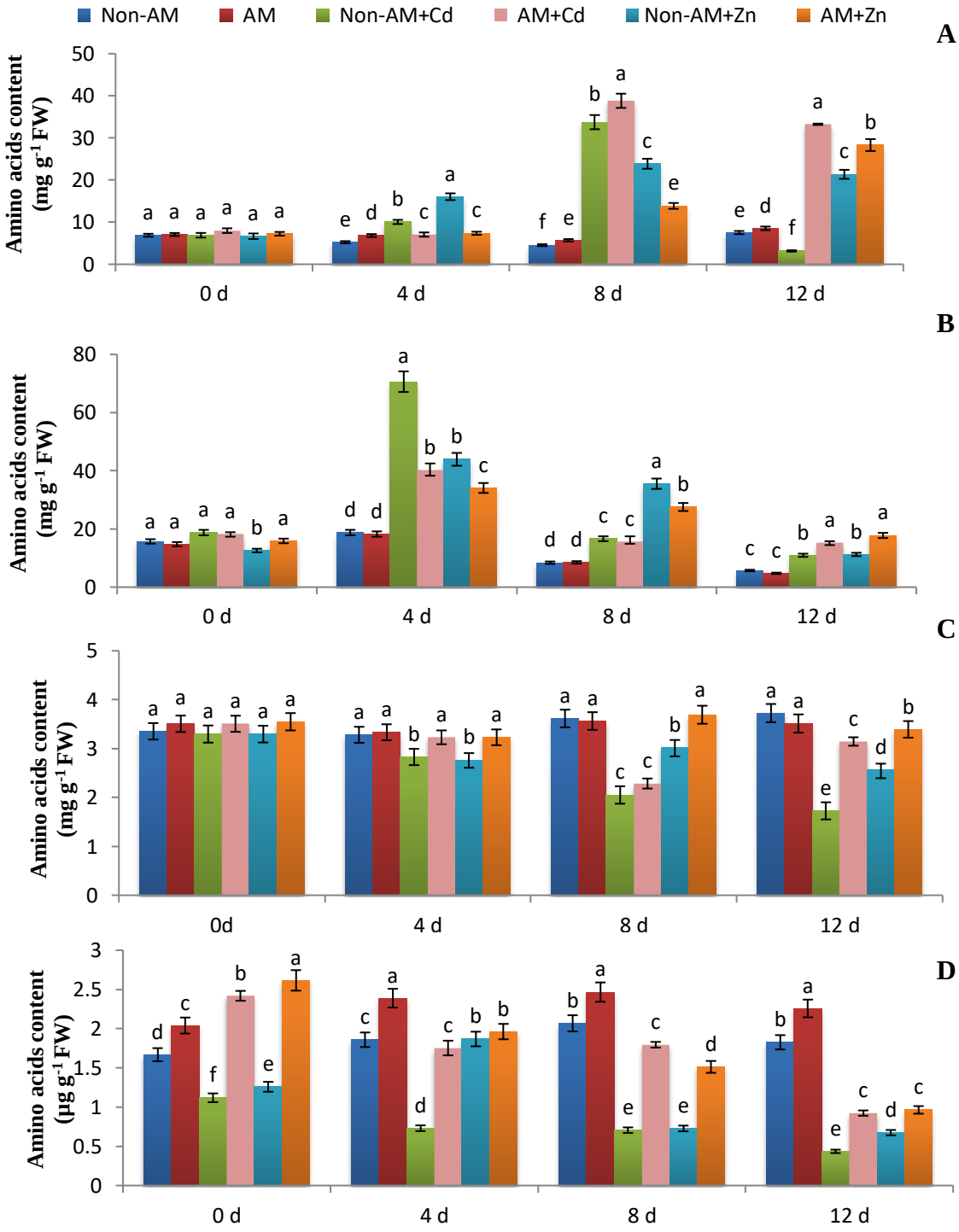

Figure 37: Total amino acid content in non-AM and AM plants A) leaves of *Z. mays*, B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

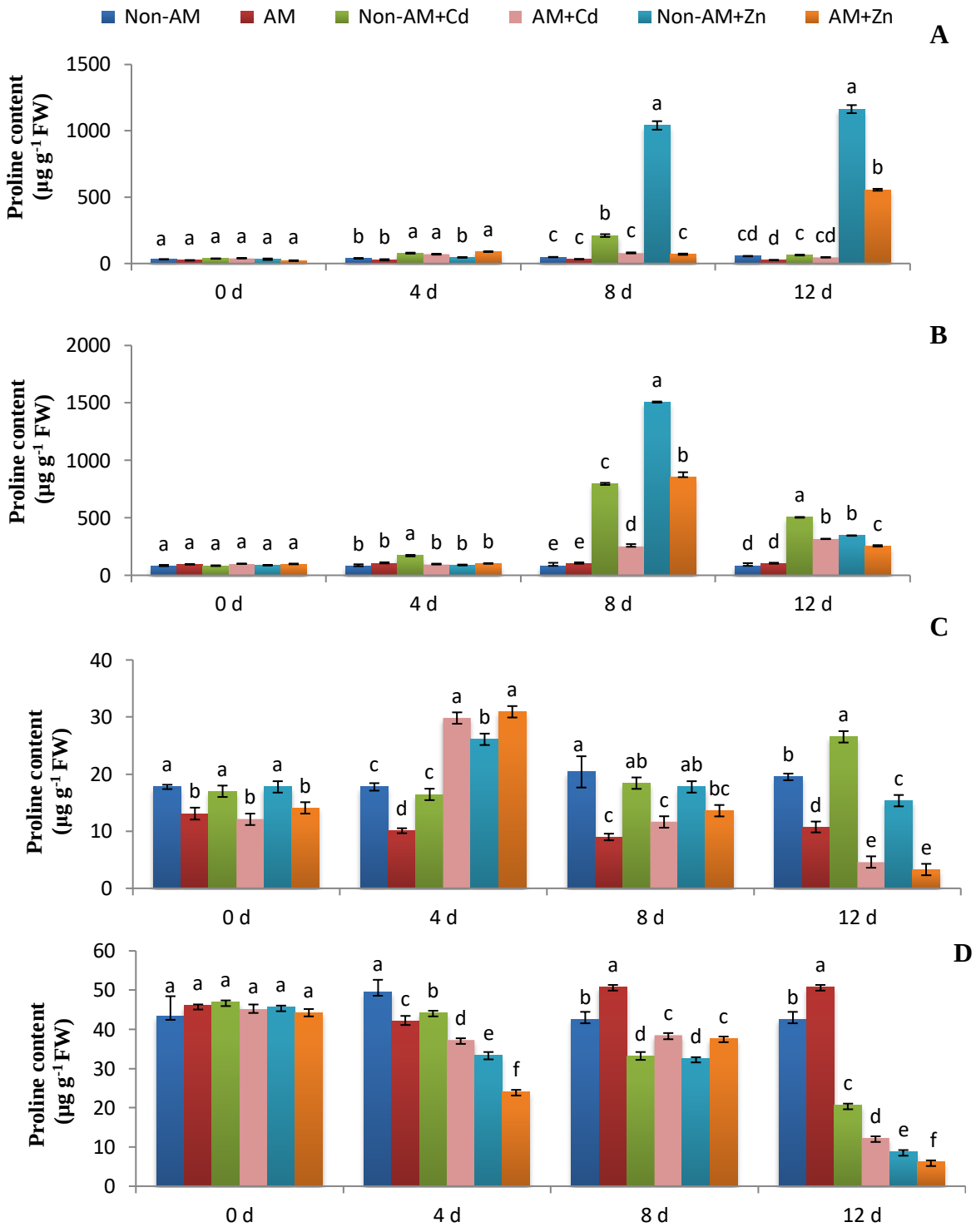

Figure 38: Proline content in non-AM and AM plants A) leaves of *Z. mays*, B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

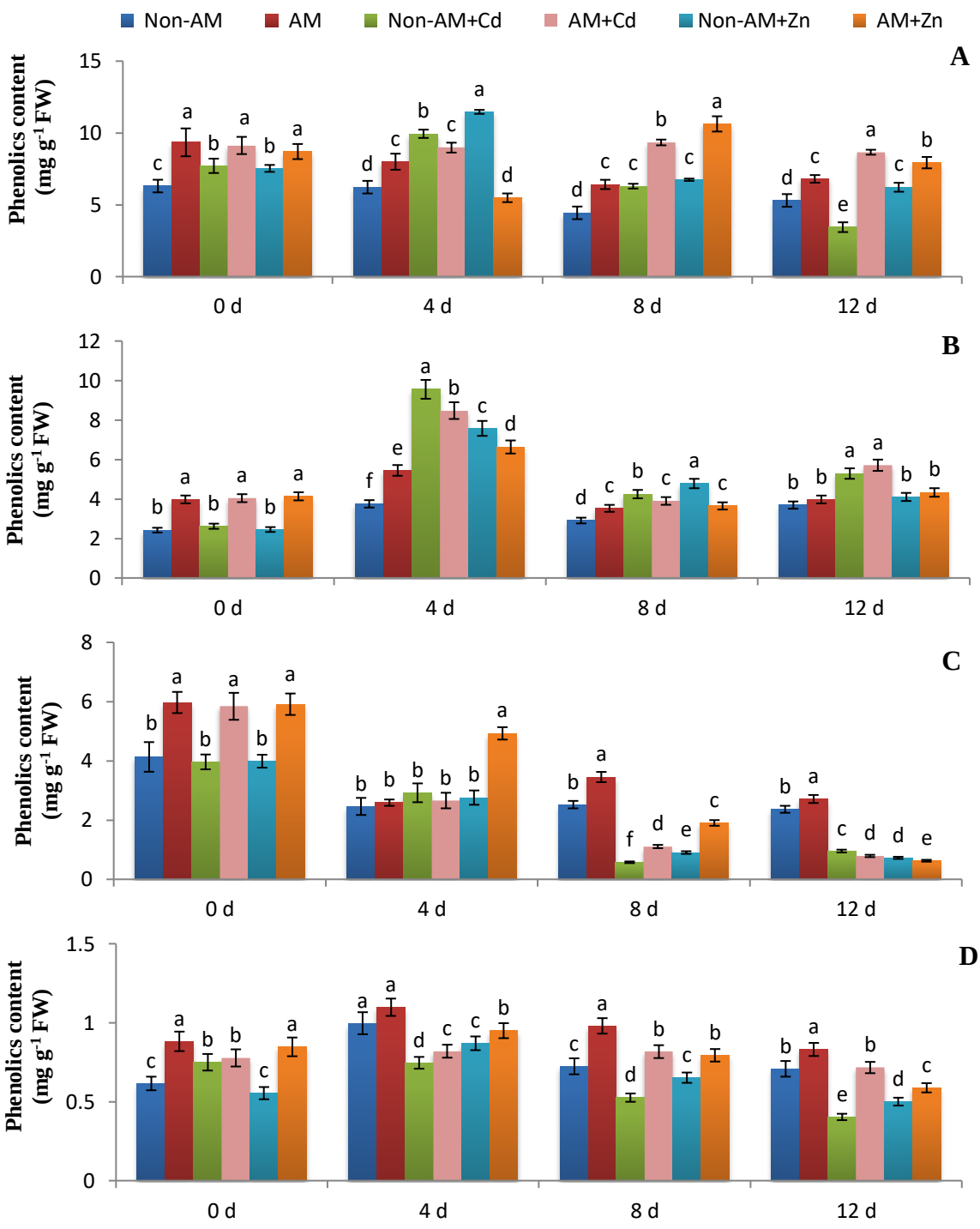

Figure 39: Phenolics content in non-AM and AM plants A) leaves of *Z. mays* B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

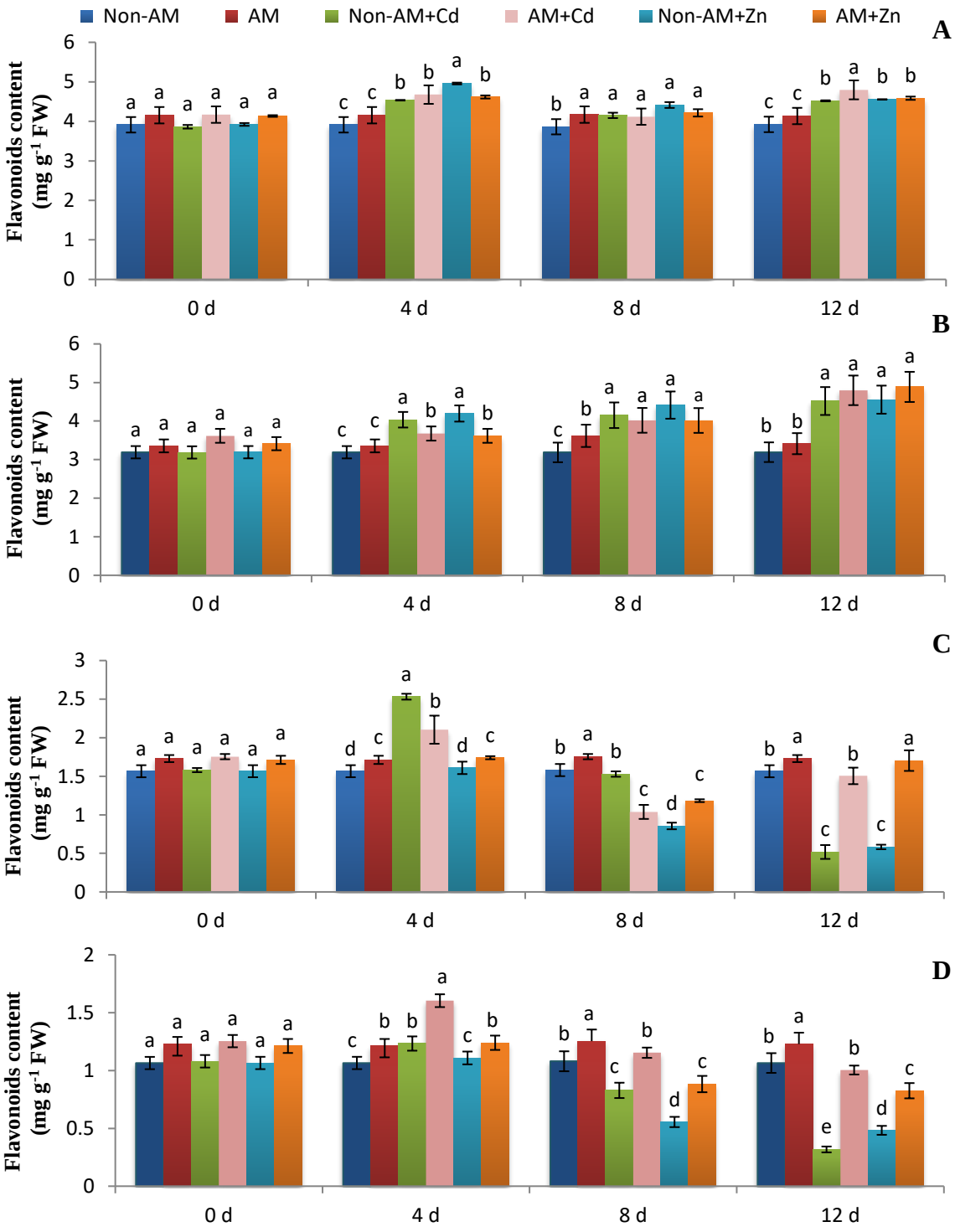

Figure 40: Flavonoids content in non-AM and AM plants of A) leaves of *Z. mays*, B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

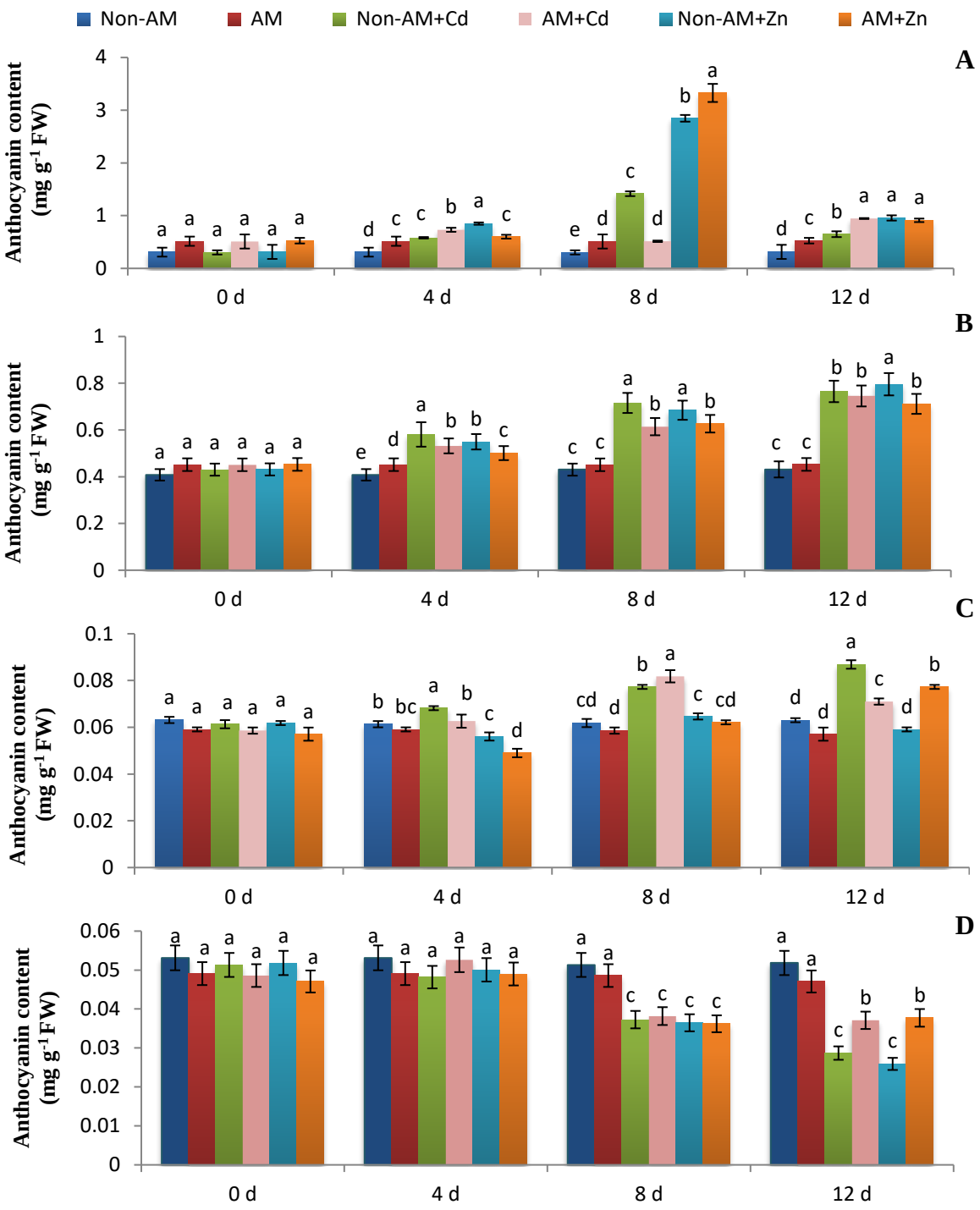

Figure 41: Anthocyanin content in non-AM and AM plants A) leaves of *Z. mays*, B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

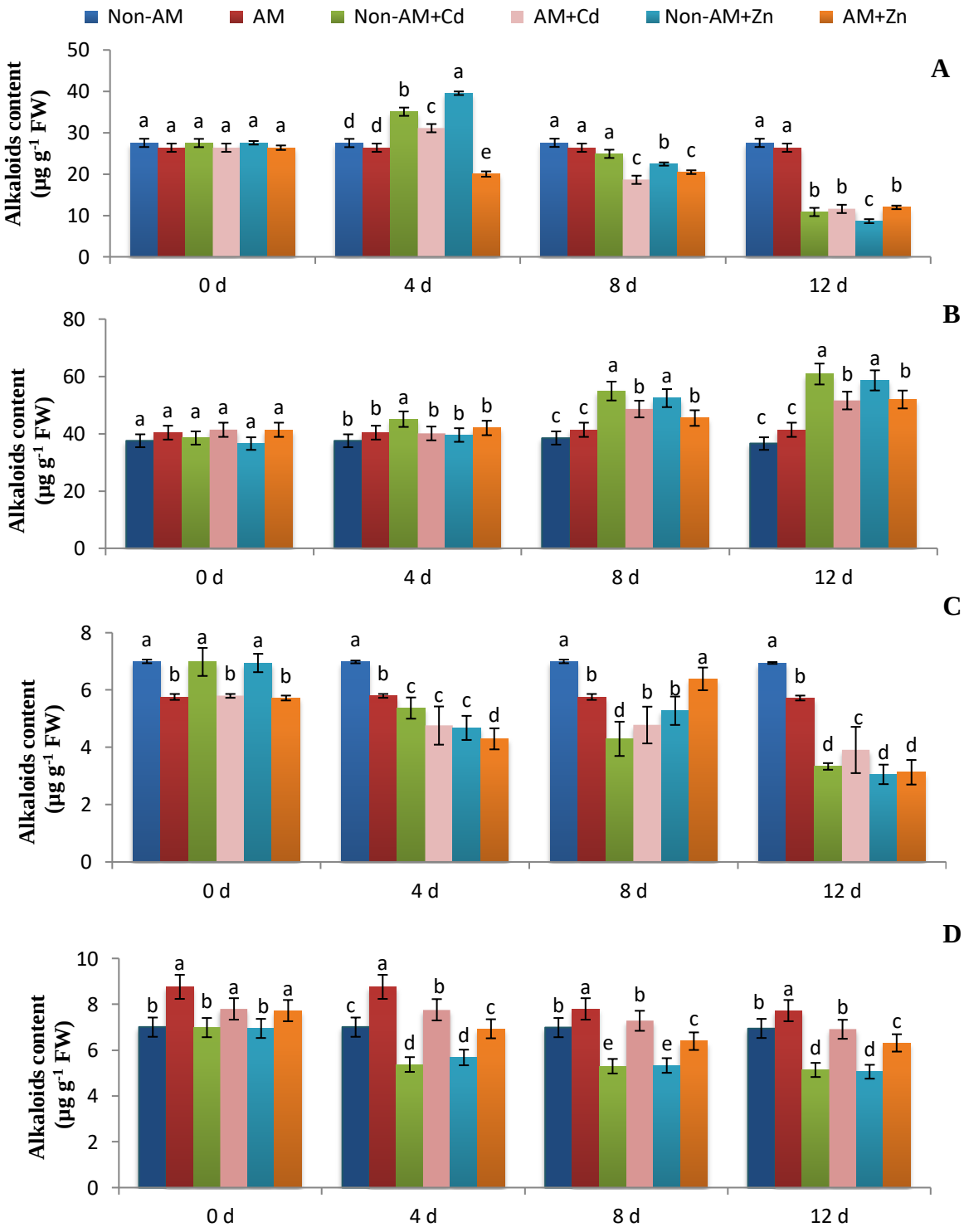

Figure 42: Alkaloids content in non-AM and AM plants A) leaves of *Z. mays* B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

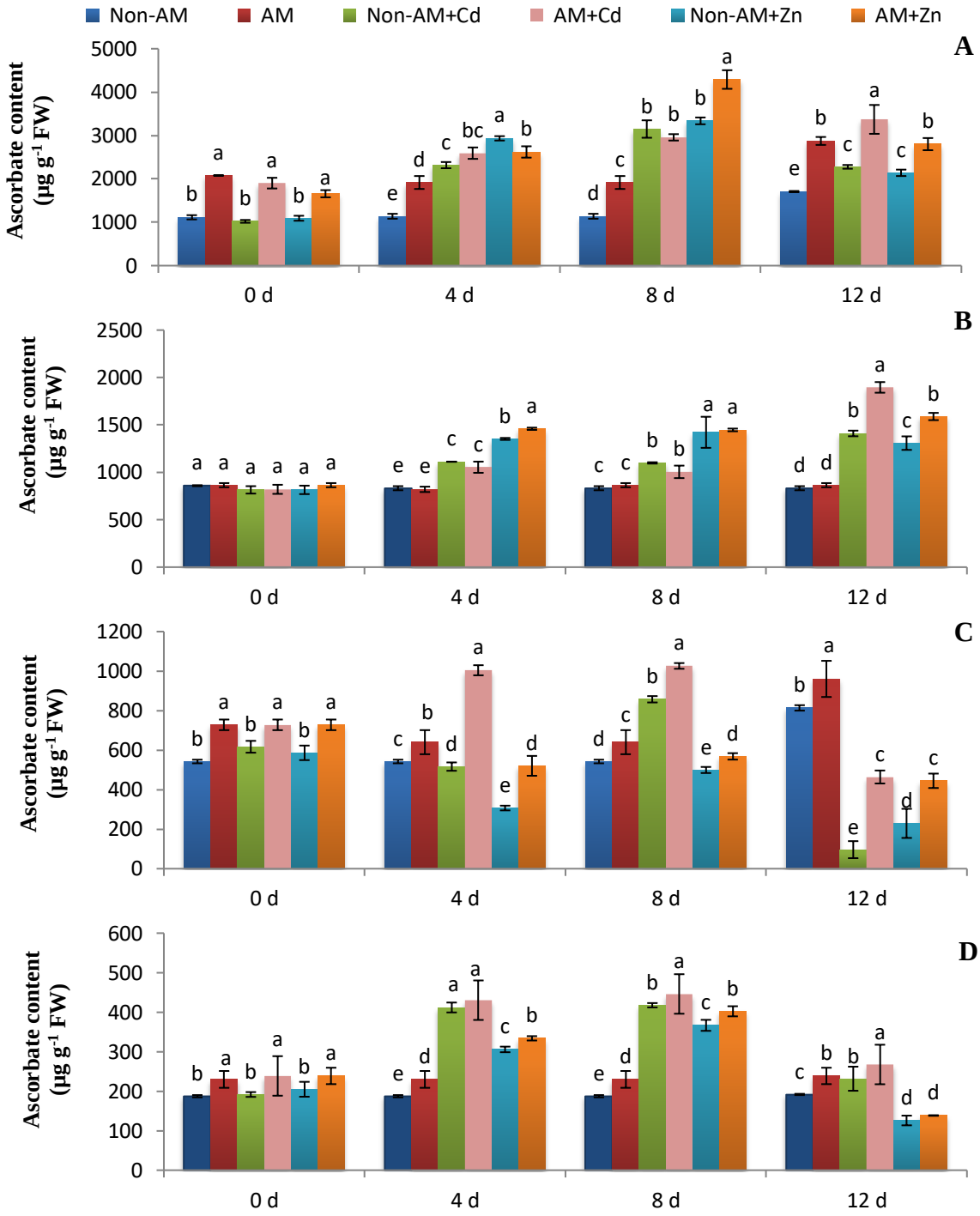

Figure 43: Ascorbate content in non-AM and AM plants of A) leaves *Z. mays* B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

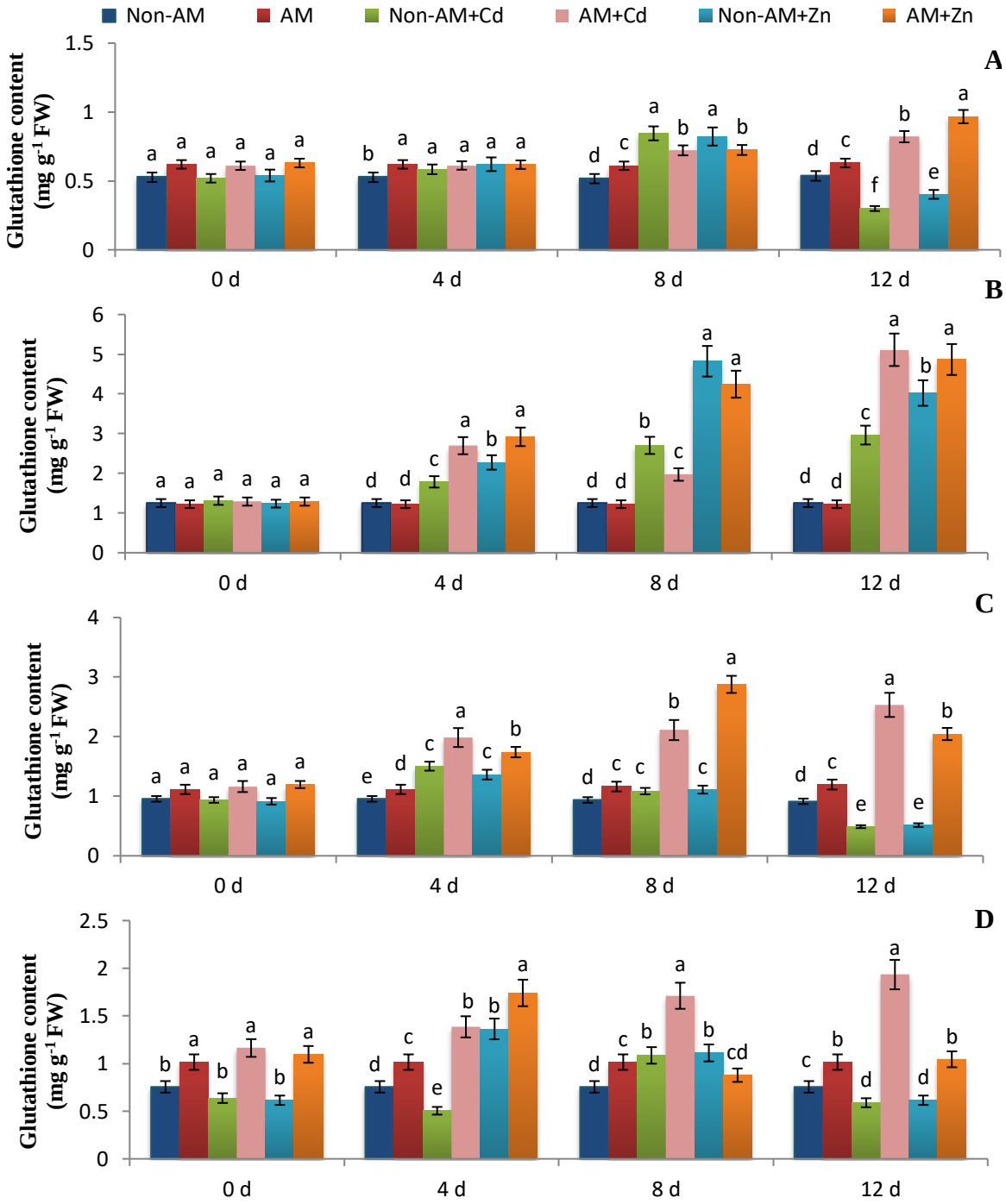

Figure 44: Glutathione content in non-AM and AM plants A) leaves of *Z. mays* B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

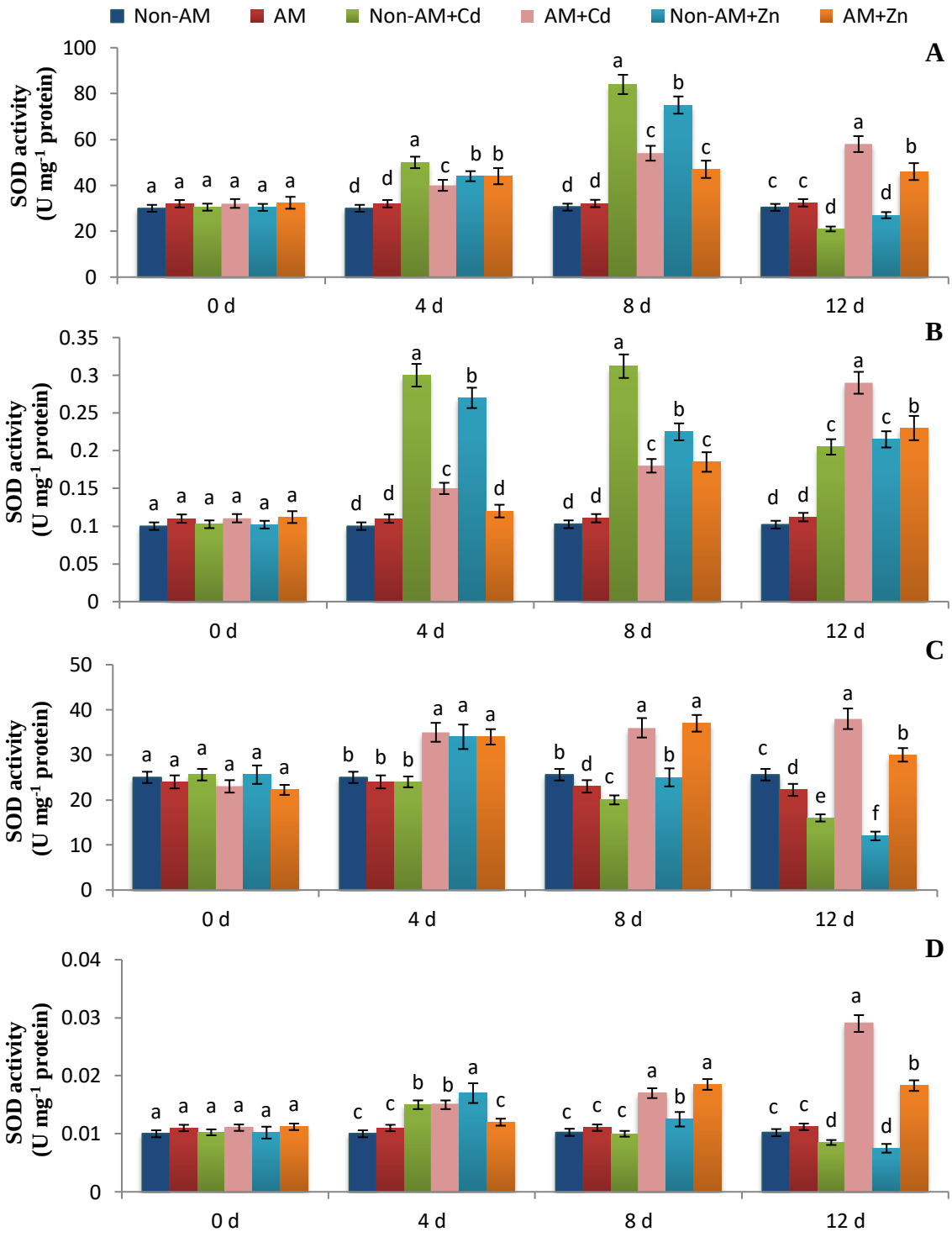

Figure 45: Superoxide dismutase activity (SOD) in non-AM and AM plants A) leaves of *Z. mays* B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

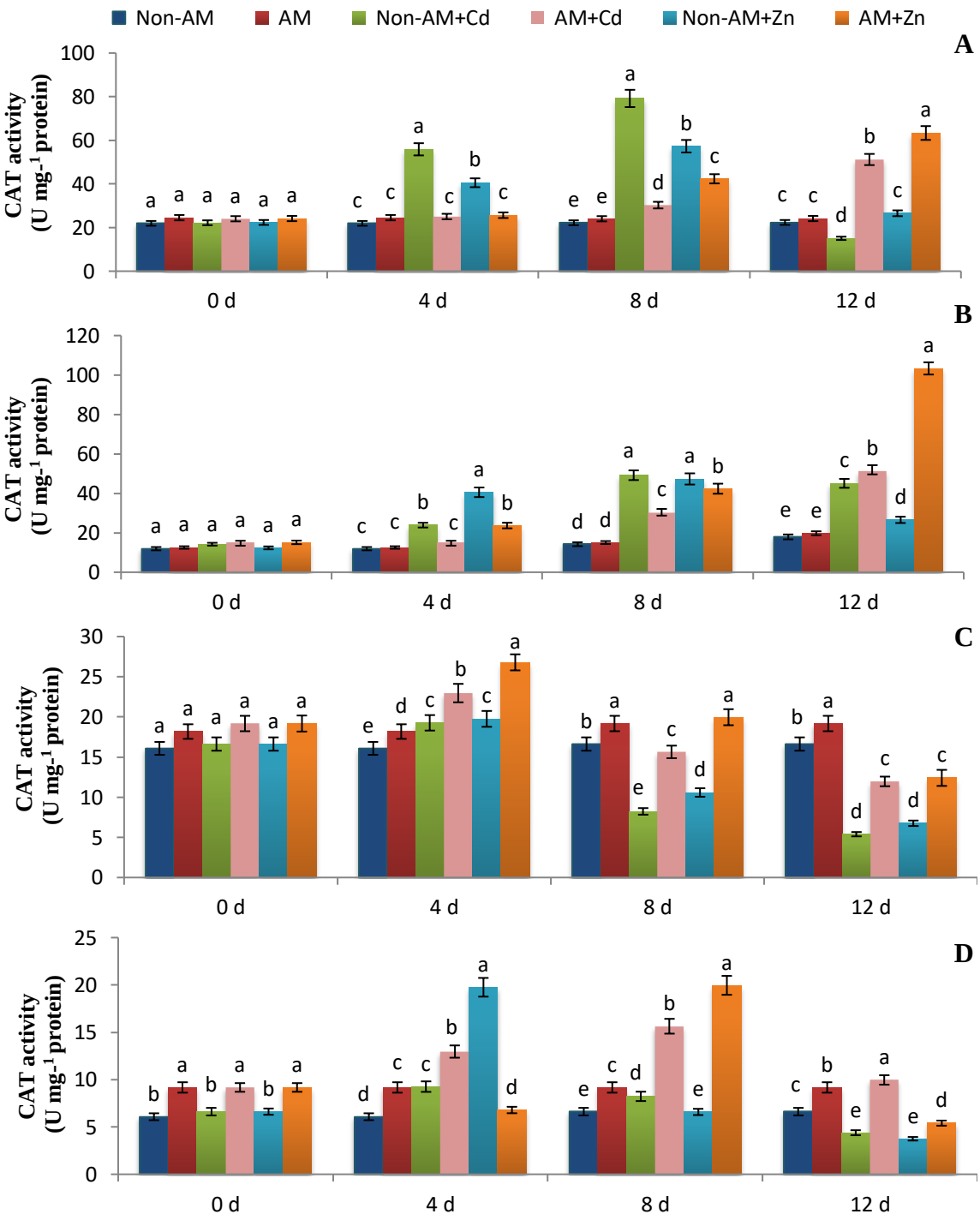

Figure 46: Catalase (CAT) activity in non-AM and AM plants A) leaves of *Z. mays* B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

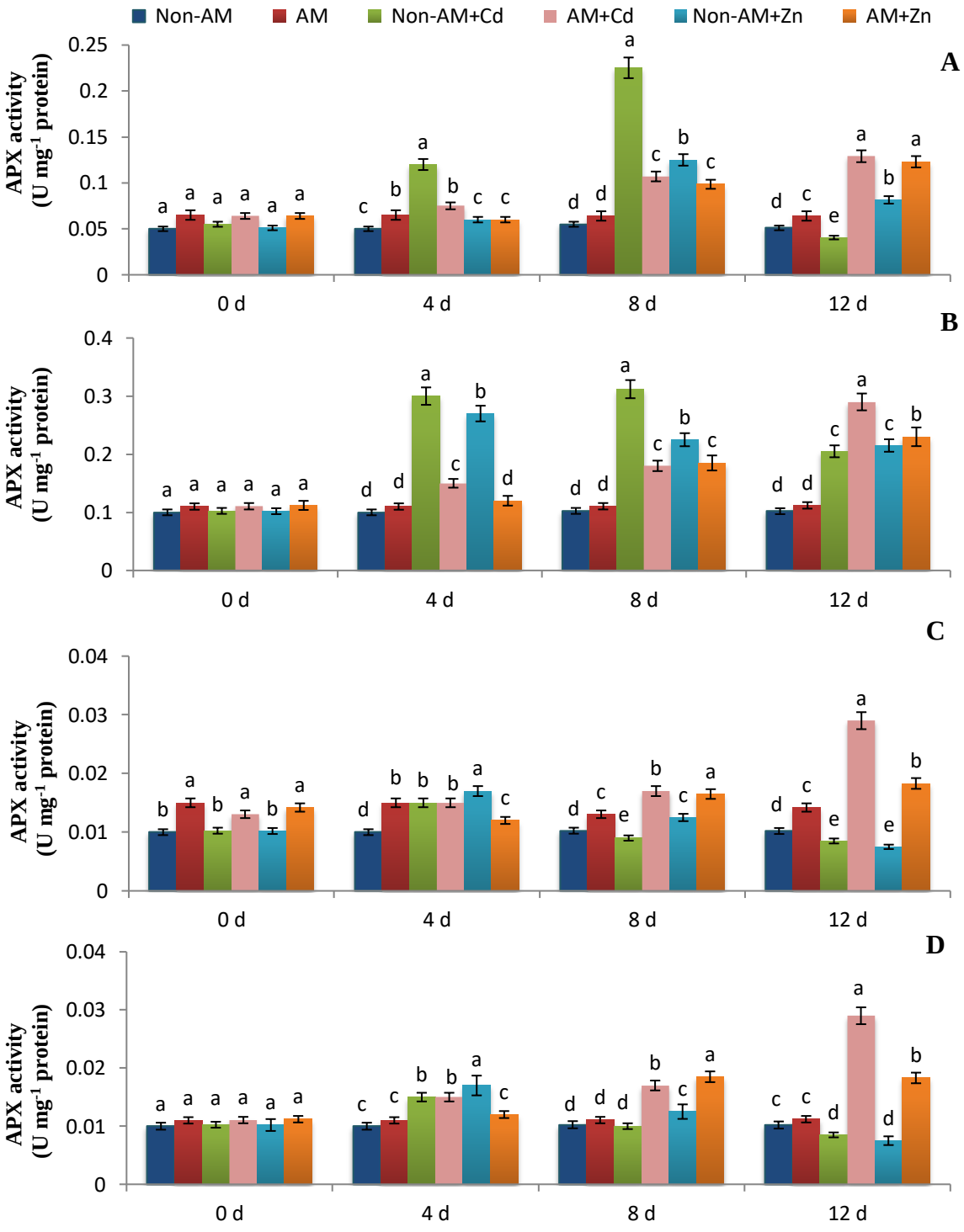

Figure 47: Ascorbate peroxidase (APX) activity in non-AM and AM plants A) leaves of *Z. mays* B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

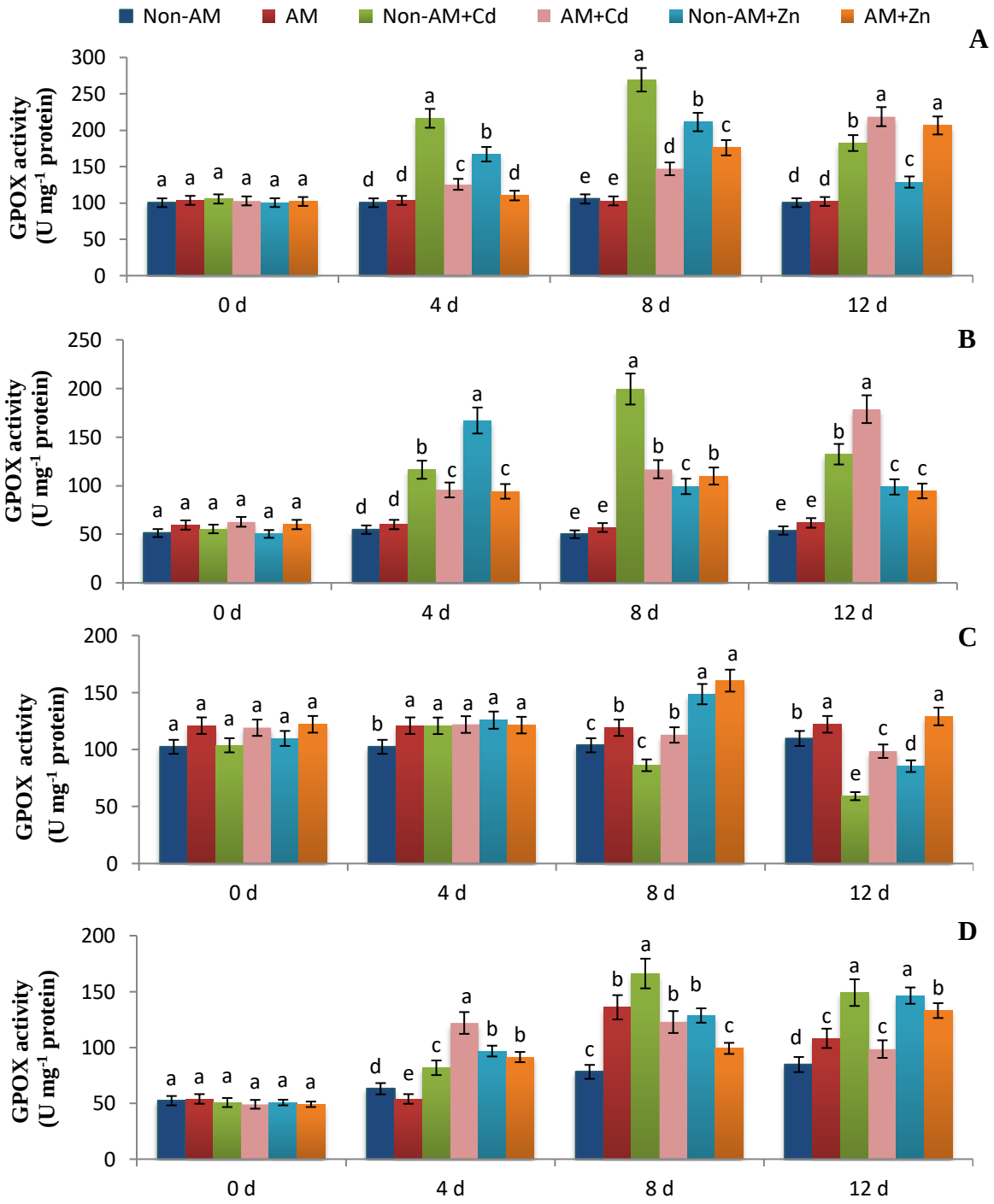

Figure 48: Guaiacol peroxidase (GPOX) activity in non-AM and AM plants A) leaves of *Z. mays* B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

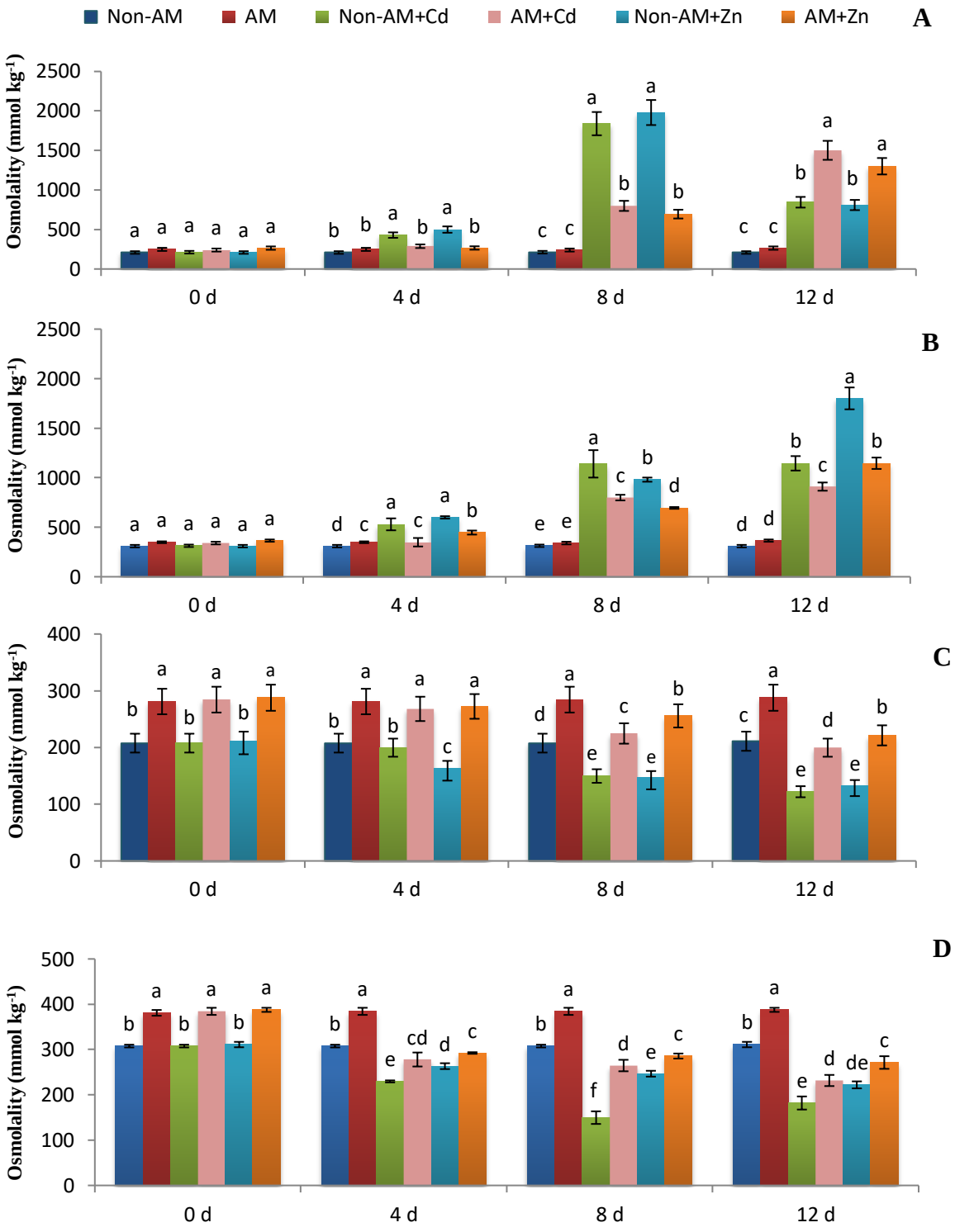

Figure 49: Osmolality in non-AM and AM plants A) leaves of *Z. mays*, B) leaves of *O. sativa* C) roots of *Z. mays*, and D) roots of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Different alphabetical letters indicate a significant difference between treatments (Duncan's test $P \leq 0.05$).

to Cd and Zn stresses but was not seen in the non treated non-AM and AM plants. At the same time, non prominent variations in intensity of peaks *viz.* 1285, 1233, 1178 cm^{-1} were observed in *O. sativa* plants.

4.5 Elemental analysis

4.5.1 Bioaccumulation of Cd and Zn

The pattern of accumulation of Cd and Zn in *Z. mays* leaves and roots were different and the roots increased the metal accumulation as compared to the leaves of non-AM and AM plants subjected to Cd and Zn stresses (Table 19). In the plants exposed to Cd stress, 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 accumulated low concentrations of Cd as compared to roots, it was 0.6594 ± 0.0305 mg g^{-1} DW and 0.6025 ± 0.0326 mg g^{-1} DW in non-AM and AM plants respectively on 8 d of exposure (Table 19). 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 in 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 higher (0.35) in AM plants. Whereas the TF of Cd was 0.13 and 0.16 in non-AM and AM plants respectively on 8d of exposure to Cd stress (Table 19).

Accumulation of Cd and Zn in *O. sativa* plants increased gradually with the increase in the period of exposure and the accumulation was higher in the roots of non-AM and AM plants subjected to Cd and Zn stresses (Table 20). In the plants exposed to Cd stress, maximum Cd accumulation was

recorded in the roots of non-AM plants (3.13 ± 0.16 mg g^{-1} DW) followed by AM plants (2.25 ± 0.11 mg g^{-1} DW). Leaves accumulated low concentrations of Cd as compared to roots, it was 0.9657 ± 0.03 mg g^{-1} DW and 0.8210 ± 0.05 mg g^{-1} DW in non-AM and AM plants respectively on 12 d of exposure (Table 20). Under the influence of Zn stress, maximum Zn accumulation was observed in the roots of AM plants (1.8604 ± 0.09 mg g^{-1} DW) as compared to non-AM plants (1.5591 ± 0.08 mg g^{-1} DW). The Zn accumulation by the leaves was very low; it was 0.2748 ± 0.03 mg g^{-1} DW in non-AM plants and 0.2309 ± 0.02 mg g^{-1} DW in AM plants. In control, TF of Zn was observed as 0.81 and 0.96 in non-AM and AM plants respectively, but under Zn toxicity it was reduced to 0.18 and 0.12 in non-AM and AM plants respectively. Whereas the TF of Cd was 0.31 and 0.36 in non-AM and AM plants respectively on 12 d of exposure to Cd stress (Table 20).

4.5.2 Pattern of cellular distribution of Cd and Zn

The pattern of the Cd and Zn distributions was significantly modified based on the tissue specificity. The leaf tissue was divided into two zones, *i.e.*, outer region consisting of epidermis and mesophyll (spectrum 1), and inner region consisting of vascular tissues (spectrum 2). The root tissue was divided into three zones *i.e.*, outer region consisting of epidermis and cortex (spectrum 1), middle region consisting of endodermis (spectrum 2), inner region consisting of pericycle and stelar tissue (spectrum 3).

The leaves of *Z. mays* and *O. sativa* had differences in the distribution of Cd and Zn ions. In *Z. mays* leaves, Cd and Zn accumulation was higher in the inner regions than mesophyll tissues and similar trend was observed in non-AM and AM plants (Fig. 54, 55, 56, 57, 58, and 59). In the case of *O. sativa*, higher Cd localization was observed in the inner regions of non-AM and AM plants. However, the Zn ions had different pattern in non-AM and AM plants (Fig. 60, 61, 62, 63, 64, and 65). In non-AM plants, maximum Zn

accumulation was observed in the outer region as compared to the inner region, but in AM plants maximum Zn accumulation was observed in the inner region.

The Zn content in the inner region of mycorrhizae associated *Z. mays* roots (not exposed to metal toxicity) increased as compared to the control (Table 21). In non-AM and AM plants of *Z. mays* roots, the maximum Cd accumulation was observed in the middle region whereas, the Zn distribution was different in non-AM and AM plants (Table 21). Under Zn stress, major portion of Zn was localized in the outer region of non-AM plants, but in AM plants it was in the middle region. In the case of Cd distribution, both non-AM and AM plants had similar rate of accumulation and it was distributed mainly in the inner and middle regions. But the outer region of AM plants had lower accumulation of Cd as compared to the non-AM plants (Fig. 66, 67, 68, 69, 70 and 71). On exposure to Zn stress, Zn content of non-AM plants increased by 105% in the inner region, but the increase was only 26% in AM plants. However, in the middle region of AM plants the accumulation of Zn increased to the extent of 50% (Table 21). In the roots of both non-AM and AM *Z. mays*, there was 66-74% reduction in the localization of Zn in the outer region.

In roots of *O. sativa*, higher Cd accumulation was observed in the inner region of Cd treated non-AM plants (Fig. 72, 73, 74, 75, 76, and 77). But in the AM plants, higher Cd accumulation was observed in the middle region. In the case of non-AM plants, Zn ions predominantly got deposited in the outer region whereas in AM plants higher Zn deposition was found in the middle region of roots and it was to the extent of 314%. Whereas in the middle region of non-AM plants, the Zn deposition was reduced as compared to the control and it was to the extent of 38% (Table 22).

4.6 Anatomical modifications

4.6.1 Anatomical modifications in leaves

The leaves of *Z. mays* and *O. sativa* have single layered upper and lower epidermis specialized with bulliform cells in the upper epidermis (Fig. 78, 79, 80, and 81). Non differentiated mesophyll tissue and the vascular bundles with 2-3 layered sclerenchymatouys bundle cap are the other important characters of *Z. mays* and *O. sativa*. Major anatomical modifications observed in the leaves of *Z. mays* and *O. sativa* exposed to Cd and Zn stresses were increases in the number and thickness of the sclerenchymatous bundle sheath cells, increases in the wall thickness of xylem elements and the blockage of xylem vessels. Under Cd stress, *Z. mays* plants (Fig. 78 and 79) significantly modified the leaf anatomy than *O. sativa* plants (Fig. 80 and 81). The loss in turgidity of mesophyll cells and the collapse of bulliform cells were clearly visualized in the SEM images of non-AM *Z. mays* leaves (Fig. 78). But theses modification were not observed in AM plants. A reduction in the phloem area as well as depositions and blockages in xylem vessels were also observed in Cd and Zn treated non-AM plants of *Z. mays*.

In the leaves of non-AM and AM *O. sativa*, thickening in the walls of the bundle sheath and xylem elements were observed, but blockage in the xylem vessels were not prominent as observed in *Z. mays* plants under Cd stress. Interestingly, the thickening of the cell walls induced by the Cd and Zn stresses was different in tissues of both *Z. mays* and *O. sativa*. In Cd treated *Z. mays* leaves, the increase in wall thickening of bundle cap cells was to the extent of 20% in both non-AM and AM plants, whereas in Zn treated *Z. mays* leaves, the xylem elements had highest level of cell wall thickening and it was up to 30% (Table 23). In the leaves of non-AM and AM *O. sativa*, the increase in the cell wall thickening of xylem elements was prominent under Cd stress. When the non-AM and AM plants were exposed to Zn stress, the increase in the wall thickening of xylem was up to 34-56% as compared to the control (Table 23).

4.6.2 Anatomical modifications in roots

Cross sections (C.S.) of both *Z. mays* and *O. sativa* roots were analysed by scanning electron micrographs. The *Z. mays* and *O. sativa* root C.S. consists of epidermis, cortex and stelar regions (Fig. 82, 83, 84, and 85). In *Z. mays*, a single layered epidermis is followed by 7-8 layered parenchymatous outer cortex and endodermis (Fig. 82). The vascular tissues consist of pericycle, phloem and xylem vessels consisting of protoxylem, early metaxylem and late metaxylem. In roots of *O. sativa*, the epidermis is 2-3 layered. The cortex is divided into outer exodermis, middle mesodermis and inner endodermis. The exodermis is sclerenchymatous and thick walled. The middle cortex was either parenchymatous or aerenchymatous and thin walled. Similar to *Z. mays*, the vascular tissues consists of pericycle, phloem and xylem vessels consisting of protoxylem, early metaxylem and late metaxylem. Both *Z. mays* and *O. sativa* under optimal condition did not show any obvious thickenings in exodermis or stelar regions.

The anatomical modifications induced in the roots of *Z. mays* and *O. sativa* on exposure to Cd and Zn stresses includes parenchymal degradation, cell wall thickening and partial obstructions in xylary elements; these modifications were more severe in *Z. mays* plants than in *O. sativa* plants (Table 23). The cortical parenchymatous cells degraded and this was the prominent response observed in *Z. mays* roots upon exposure to Cd and Zn stresses (Fig. 82).

The parenchymatous tissues in the stelar region were completely damaged, and the cortical parenchyma was shrunken in roots of Cd treated *Z. mays* plants (Fig. 82C and D, 83C and D). In the roots of Zn-treated plants, the inner cortical parenchyma was significantly damaged, the outer cortical parenchyma cells became larger in size, and there was an increase in the intercellular space (Fig. 82 E and F). The non-AM plants of *Z. mays* subjected

to Cd and Zn stresses exhibited high rate of parenchyma tissue degradation as compared to the Cd and Zn treated AM plants. Under the influence of Cd and Zn stresses, the cortical regions of *O. sativa* did not exhibit any significant anatomical modifications (Fig. 84 and 85).

The most remarkable modification observed in response to Cd and Zn stresses in *O. sativa* and *Z. mays* was wall thickening in the root xylem as well as in the inner transverse wall of the endodermal cells (Table 23). Upon exposure to Cd stress, the thickening of the inner transverse wall of the endodermal cells in the root of non-AM plants of *Z. mays* enhanced by 68% as compared to the control, but the increase in the wall thickening of AM plants was only to the extent of 19%. The thickening in the walls of the xylem elements was even more pronounced in the roots of non-AM plants exposed to Zn stress, which was 105% thicker than the control, while in AM plants cell wall thickening increased up to 95%. However, in *O. sativa* roots, Cd and Zn stresses induced thickening of epidermal cells. The increase was up to 282% in non-AM and 270% in AM plants exposed to Cd and Zn stresses. The walls of xylem elements were thickened by only 50-59% in non-AM and AM plants as compared to the control. The cell walls of the endodermis in roots did not show any significant modifications under Cd and Zn stresses in *O. sativa* (Table 23).

Depositions of electron dense particles were observed in the stelar regions of Zn and Cd treated *Z. mays* and *O. sativa*. This depositions were prominently observed in the stelar regions of non-AM plants as compared to AM plants (Fig. 83 and 85).

4.7 Effect of heavy metal stress on mycorrhizal colonization

In *Z. mays* plants, on 12 d of imposing HM stress, a significant reduction in the distribution frequency of arbuscules was observed in control plants as compared to the arbuscular frequency on 4 d, but number of arbuscules was not changed in plants exposed to Cd and Zn stresses as compared to arbuscular

frequency of control plants on 4 d of stress. The arbuscular frequency in *Z. mays* treated with Cd and Zn stresses increased to 53-61% as compared to the non treated mycorrhizal plants (Table 24). Frequency of vesicles was also increased during the HM stress, under Cd and Zn stresses, the increase was up to 10%. More than the frequency, the diameter of vesicles was increased (18%) on 8 d of Cd and Zn stresses. Moreover, the spore development also significantly increased during imposition of heavy metal stress and the increase was up to 28-35% on 12 d of exposure to Cd and Zn stresses (Table 24).

In the case of *O. sativa*, arbuscular frequency was decreased in control plants on 12 d as compared to the arbuscular frequency on 4 d, but there was no reduction observed in the plants exposed to Cd and Zn stresses. The arbuscular frequency was increased to 9-10% in Cd and Zn treated plants on 12 d as compared to the arbuscular frequency of non treated mycorrhizal plants. The frequency of vesicles was also increased (8-10%) under the influence of Cd and Zn stresses. Moreover, the diameter of the vesicles were also increased on exposure to both metal stresses, and the increase was 45 and 190% respectively in roots exposed Cd and Zn stresses. Similarly, the spore development also increased by 11-22% on 12 d of exposure to Cd and Zn stresses (Table 25).

4.8 Tolerance index (TI) and mycorrhizal dependency (MD)

The TI of *Z. mays* exposed to Cd and Zn stresses were 62 and 67% respectively, but the TI of *O. sativa* was higher, and it was 82 and 101%. Whereas in mycorrhizae associated *Z. mays* on exposure to Cd and Zn stresses, the TI increased to 77 and 91%, but in *O. sativa* it was up to 101-117% (Table 26).

The MD had an alternative trend, where *Z. mays* had a higher mycorrhizal dependency under HMs stress. The MD of *Z. mays* and *O. sativa* was 52 ± 0.895 and 18 ± 0.182 in non treated plants, respectively. Whereas, the MD of *Z. mays* and *O. sativa* was 61- 63% and 24-88% respectively under Cd and Zn stresses (Table 26).

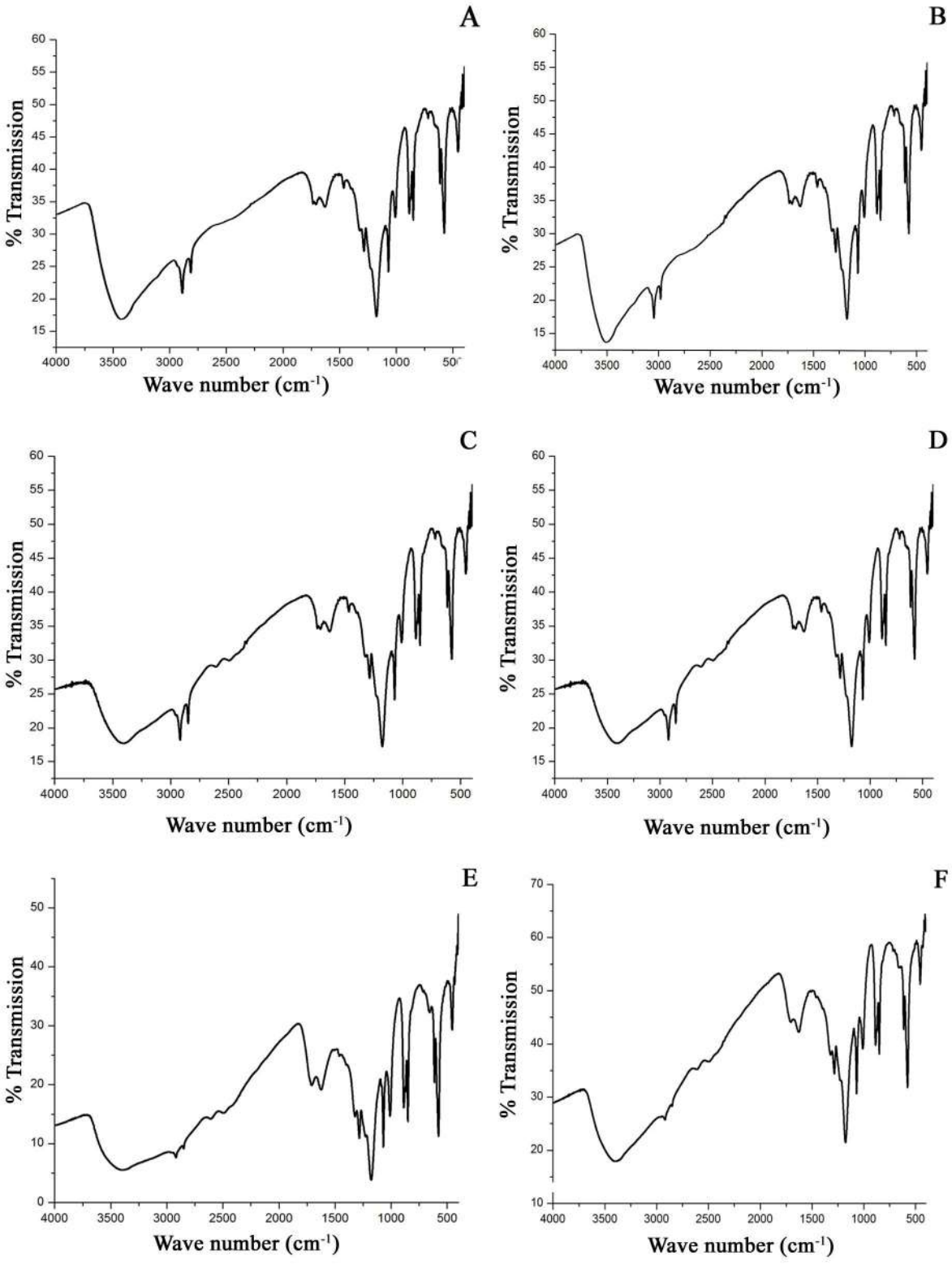

Figure 50: FTIR spectra of lignin isolated from the leaves of non-AM and AM *Z. mays* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd D) AM+Cd, E) non-AM+Zn and F) AM+Zn.

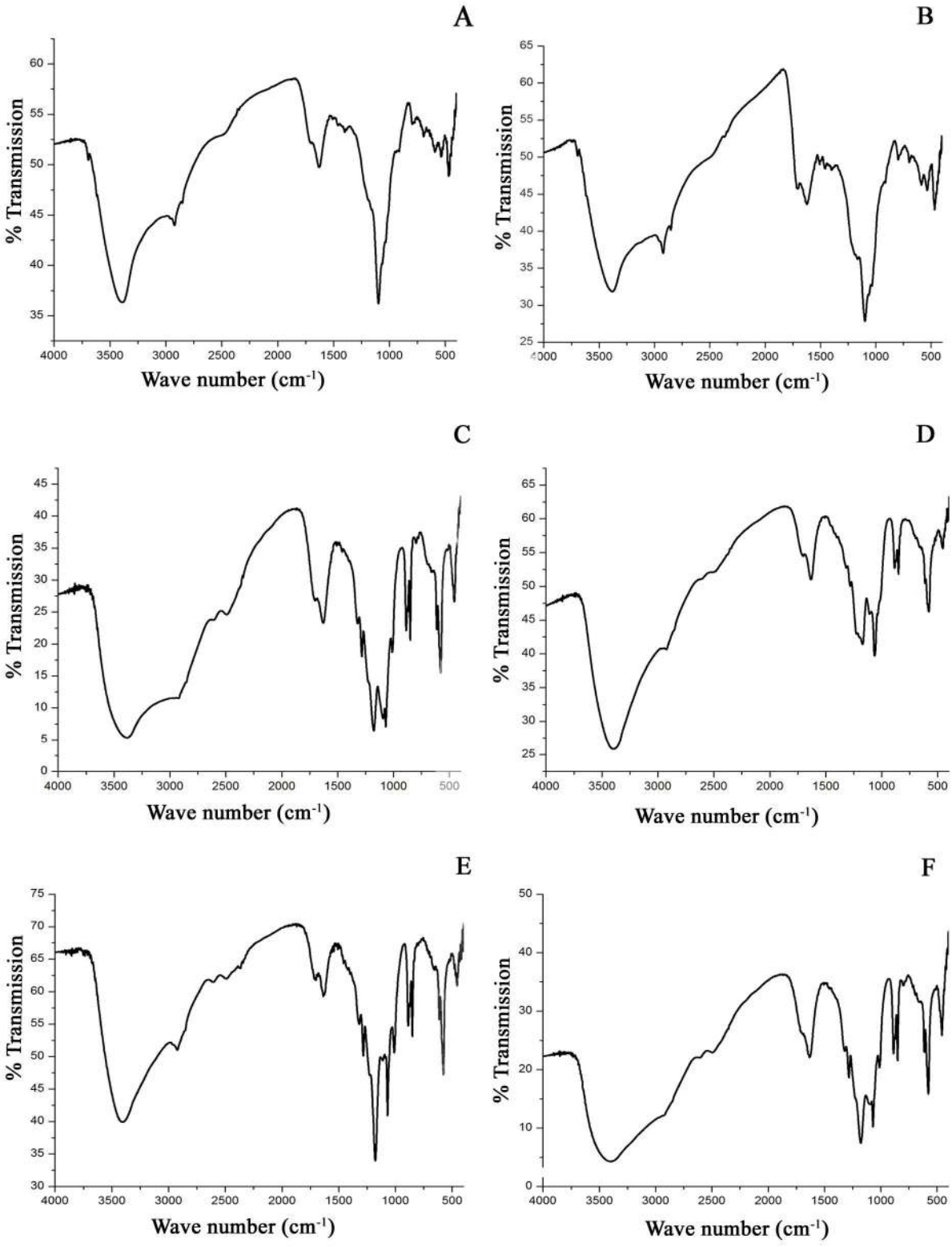

Figure 51: FTIR spectra lignin isolated from the roots of non-AM and AM *Z. mays* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd D) AM+Cd, E) non-AM+Zn and F) AM+Zn.

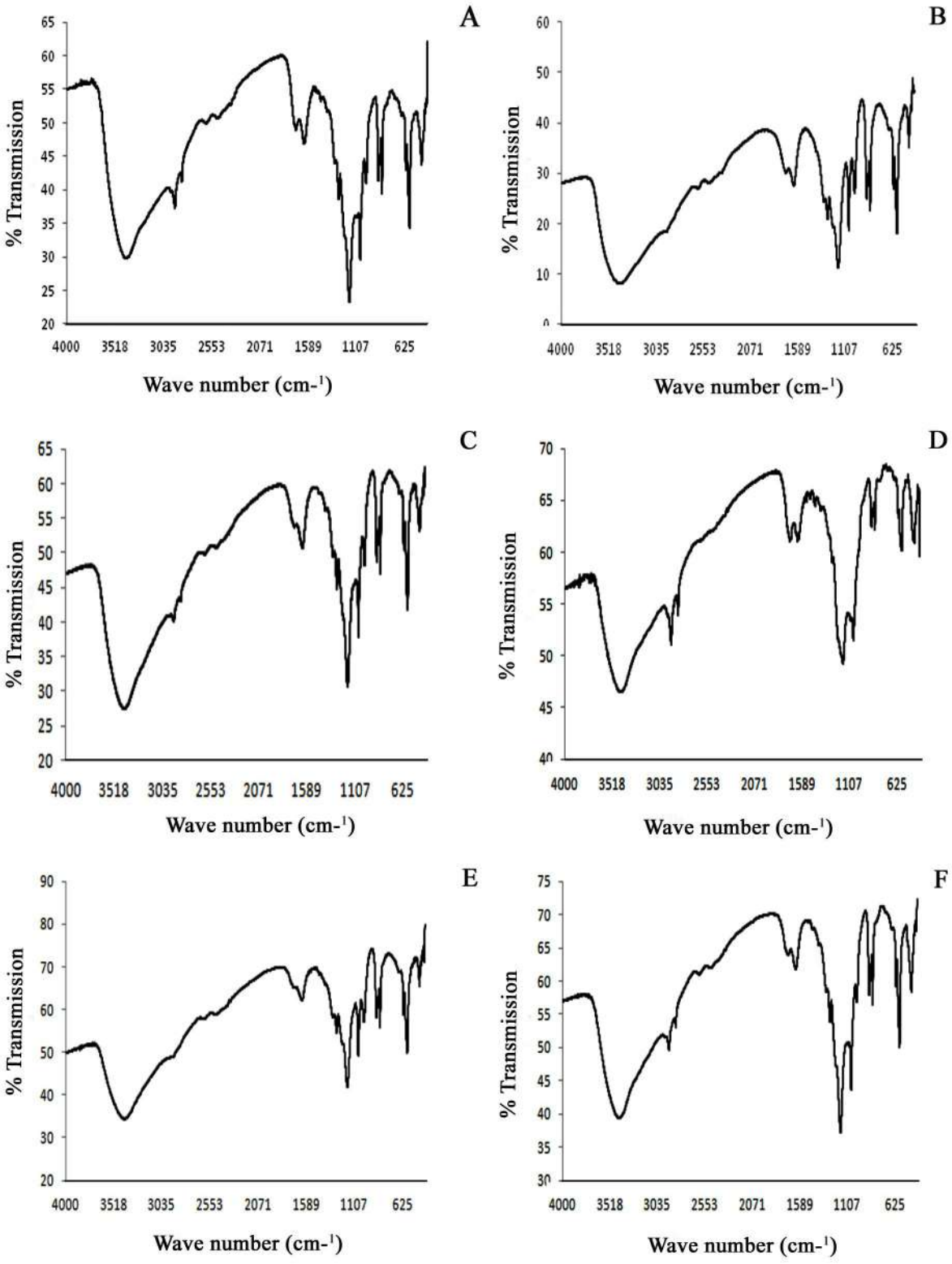

Figure 52: FTIR spectra of lignin isolated from the leaves of non-AM and AM *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd D) AM+Cd, E) non-AM+Zn and F) AM+Zn.

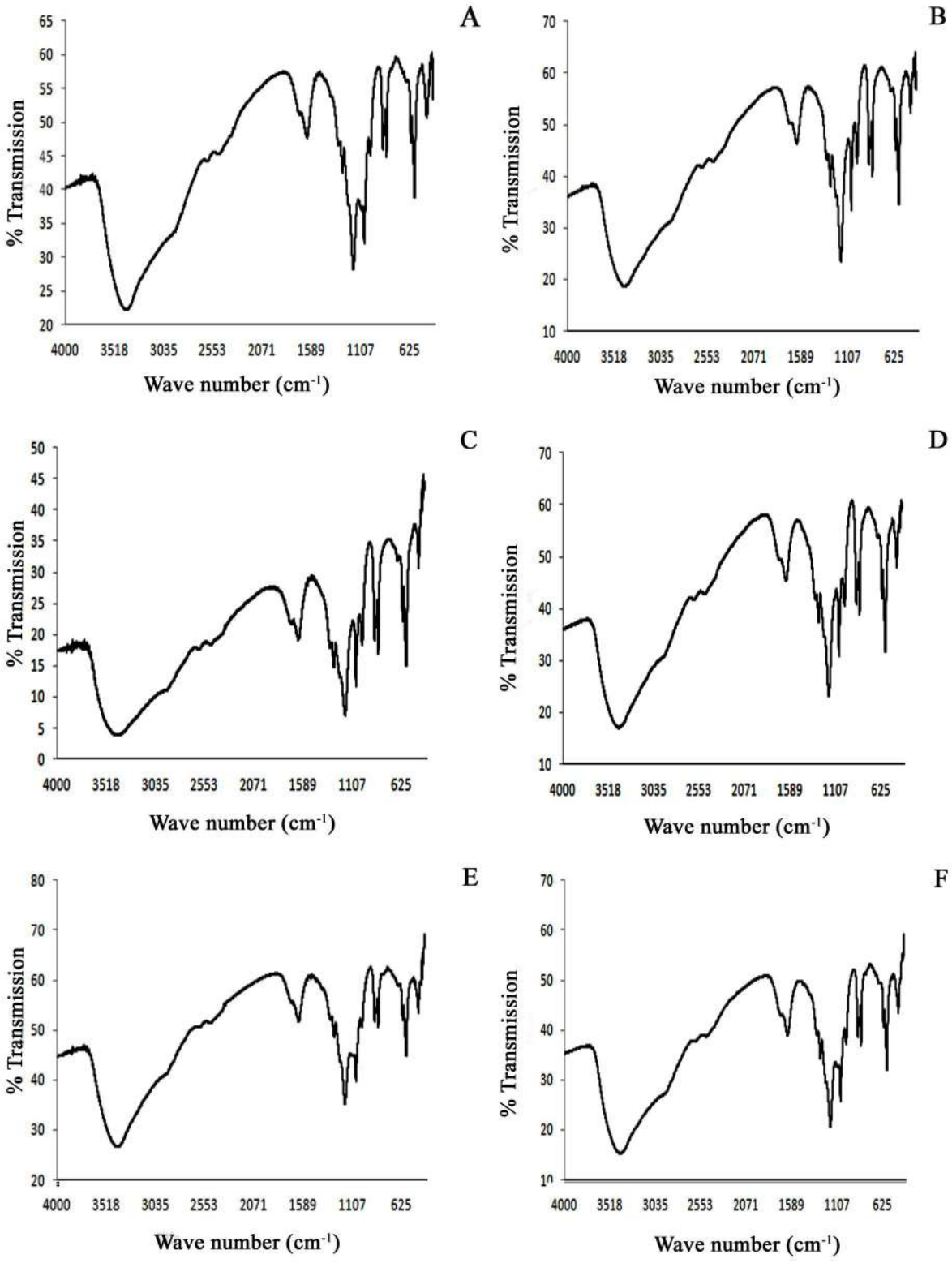

Fig. 53: FTIR spectra of lignin isolated from the roots of non-AM and AM *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd D) AM+Cd, E) non-AM+Zn and F) AM+Zn.

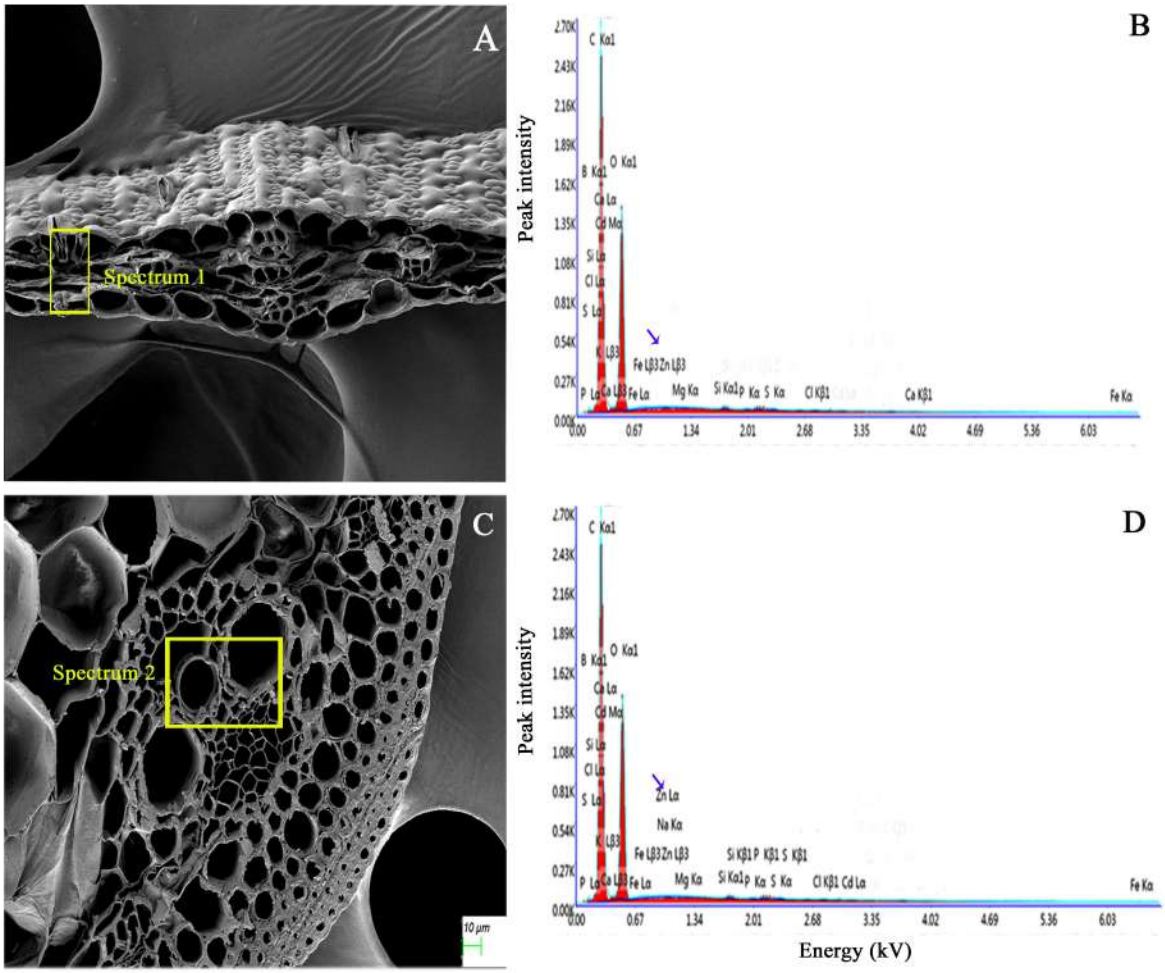

Figure 54: SEM images (A and C) and EDX spectra (B and D) of non-AM plants; two different micro spots were represented as spectrum 1, and spectrum 2 in the leaves of *Z. mays*.

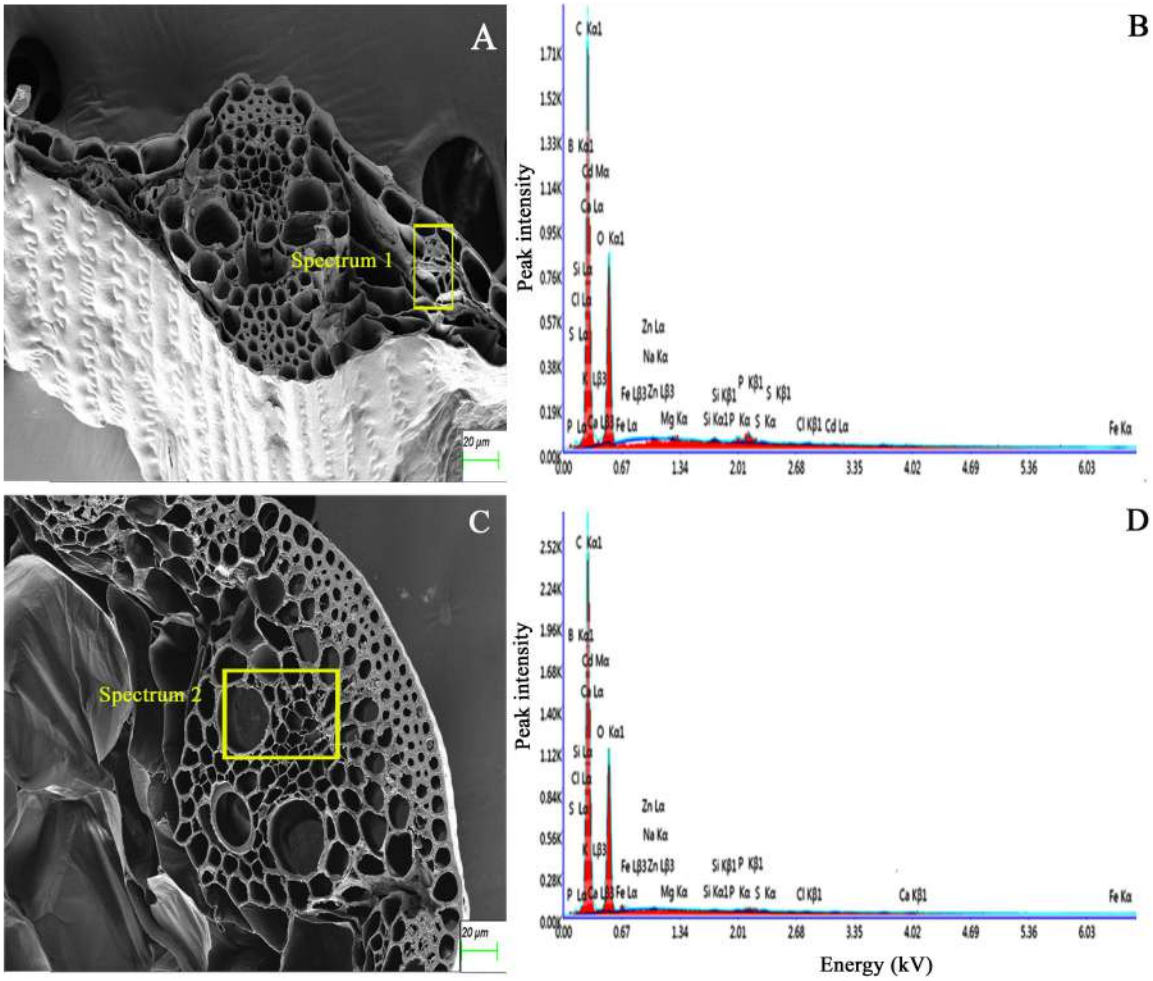

Figure 55: SEM images (A and C) and EDX spectra (B and D) of AM plants; two different micro spots were represented as spectrum 1, and spectrum 2 in the leaves of *Z. mays*.

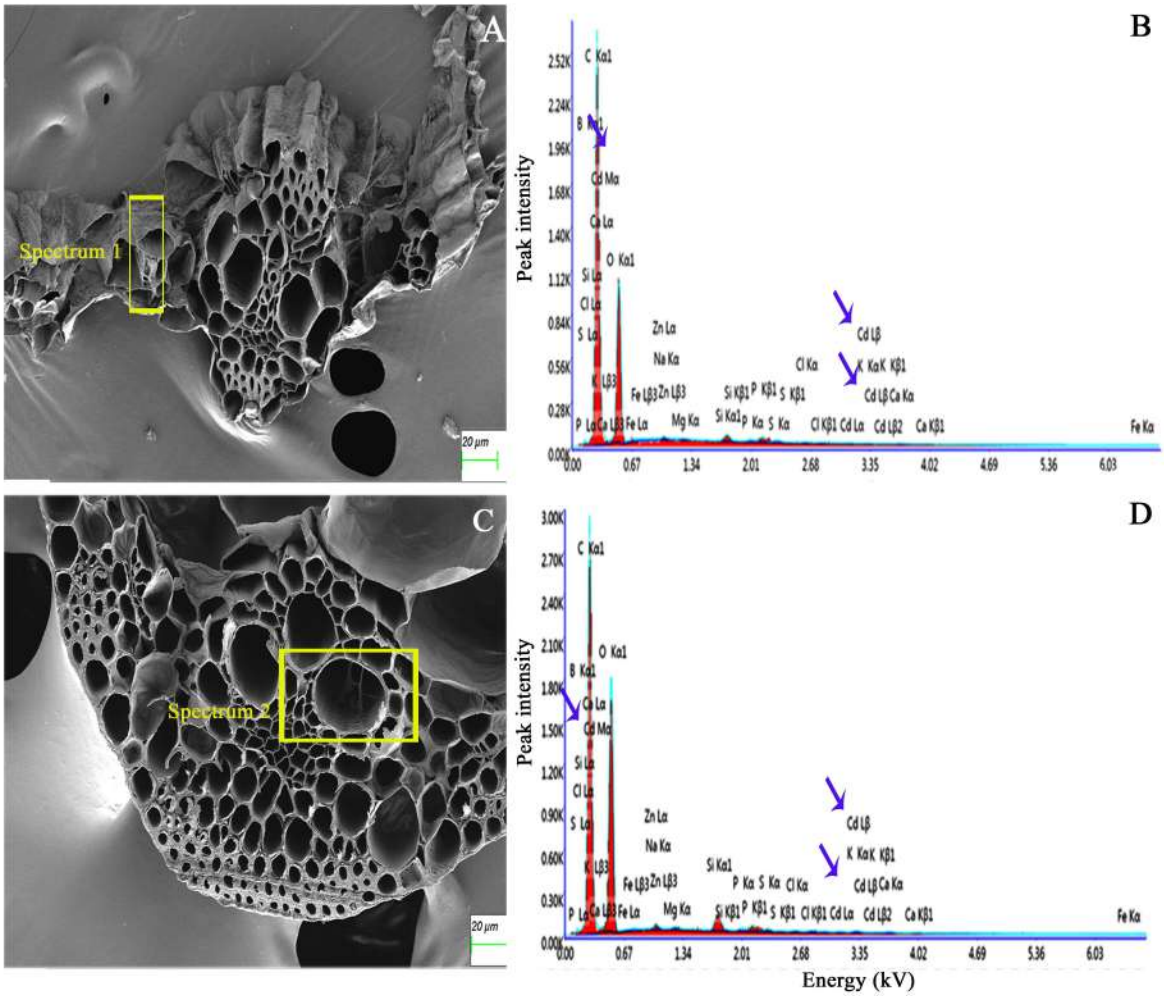

Figure 56: SEM images (A and C) and EDX spectra (B and D) of non-AM plants exposed to Cd stress; two different micro spots were represented as spectrum 1, and spectrum 2 in the leaves of *Z. mays*.

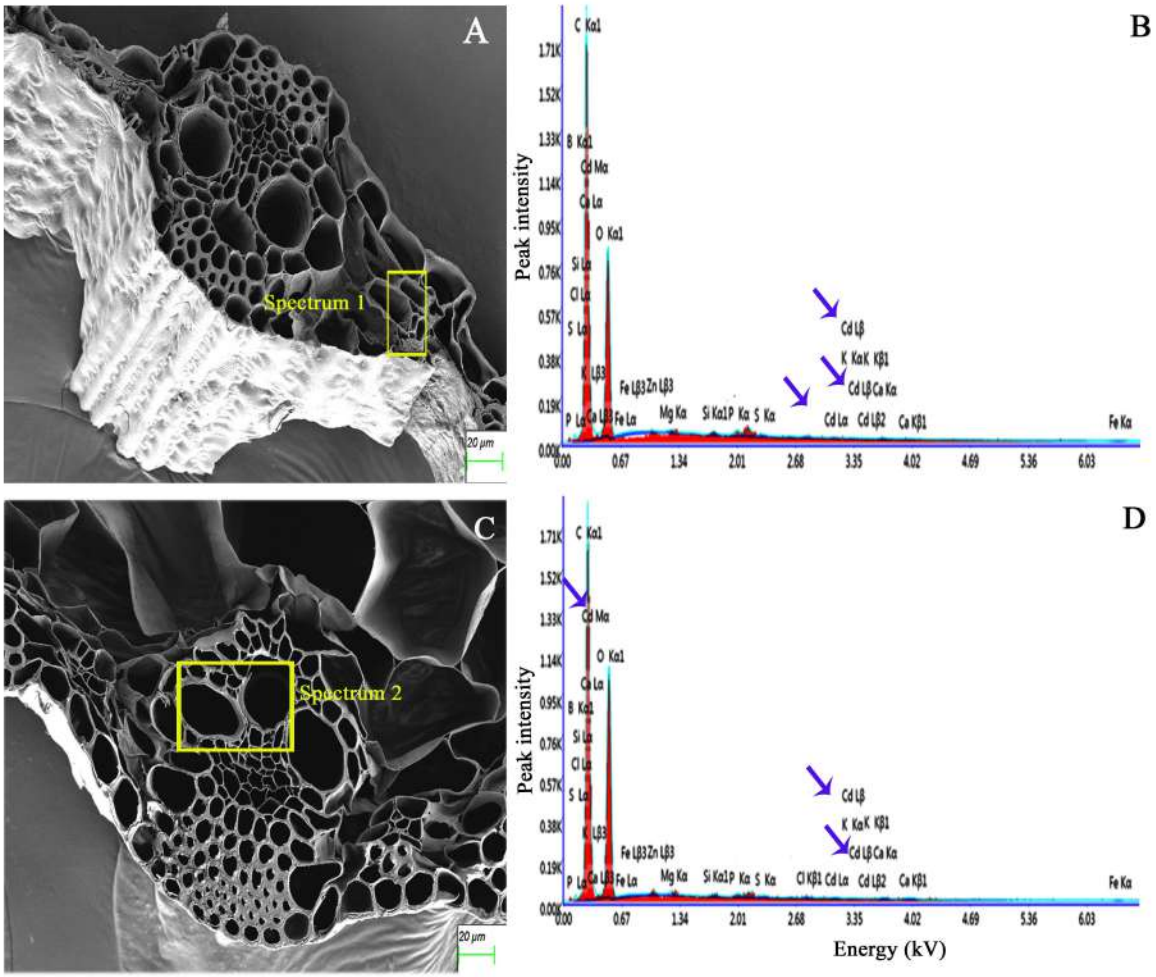

Figure 57: SEM images (A and C) and EDX spectra (B and D) of AM plants exposed to Cd stress; two different micro spots were represented as spectrum 1, and spectrum 2 in the leaves of *Z. mays*.

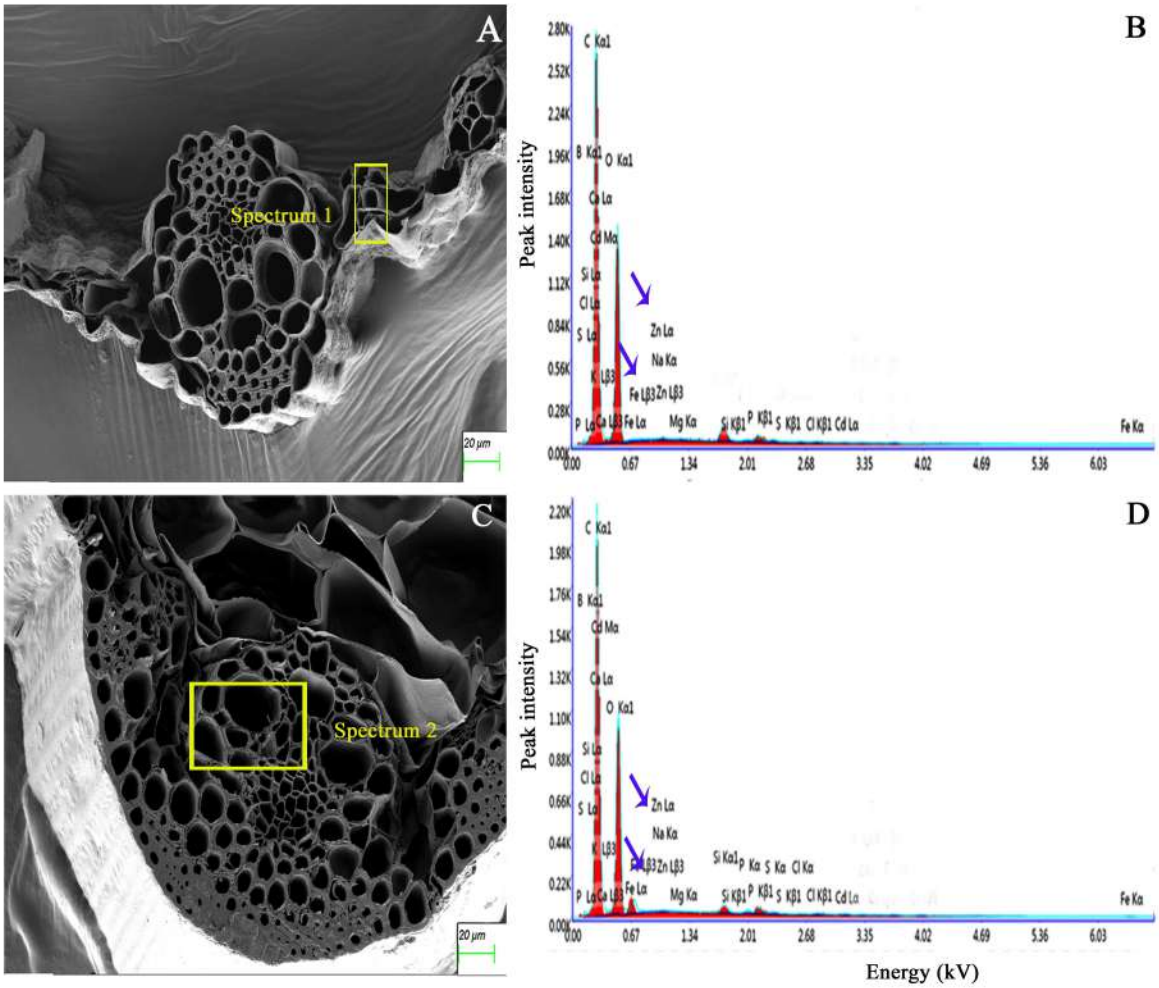

Figure 58: SEM images (A and C) and EDX spectra (B and D) of non-AM plants exposed to Zn stress; two different micro spots were represented as spectrum 1, and spectrum 2 in the roots of *Z. mays*.

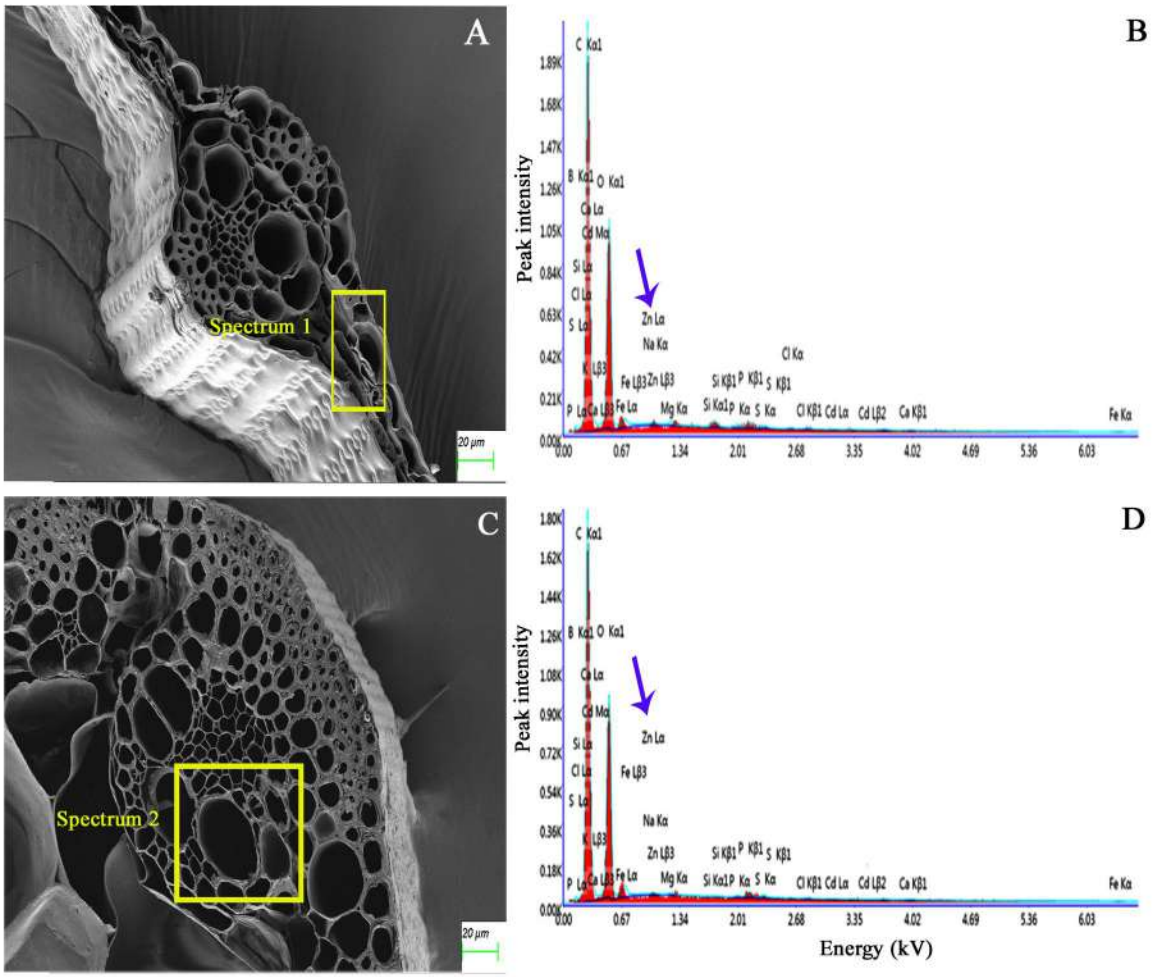

Figure 59: SEM images (A and C) and EDX spectra (B and D) of AM plants exposed to Zn stress; two different micro spots were represented as spectrum 1, and spectrum 2 in the leaves of *Z. mays*.

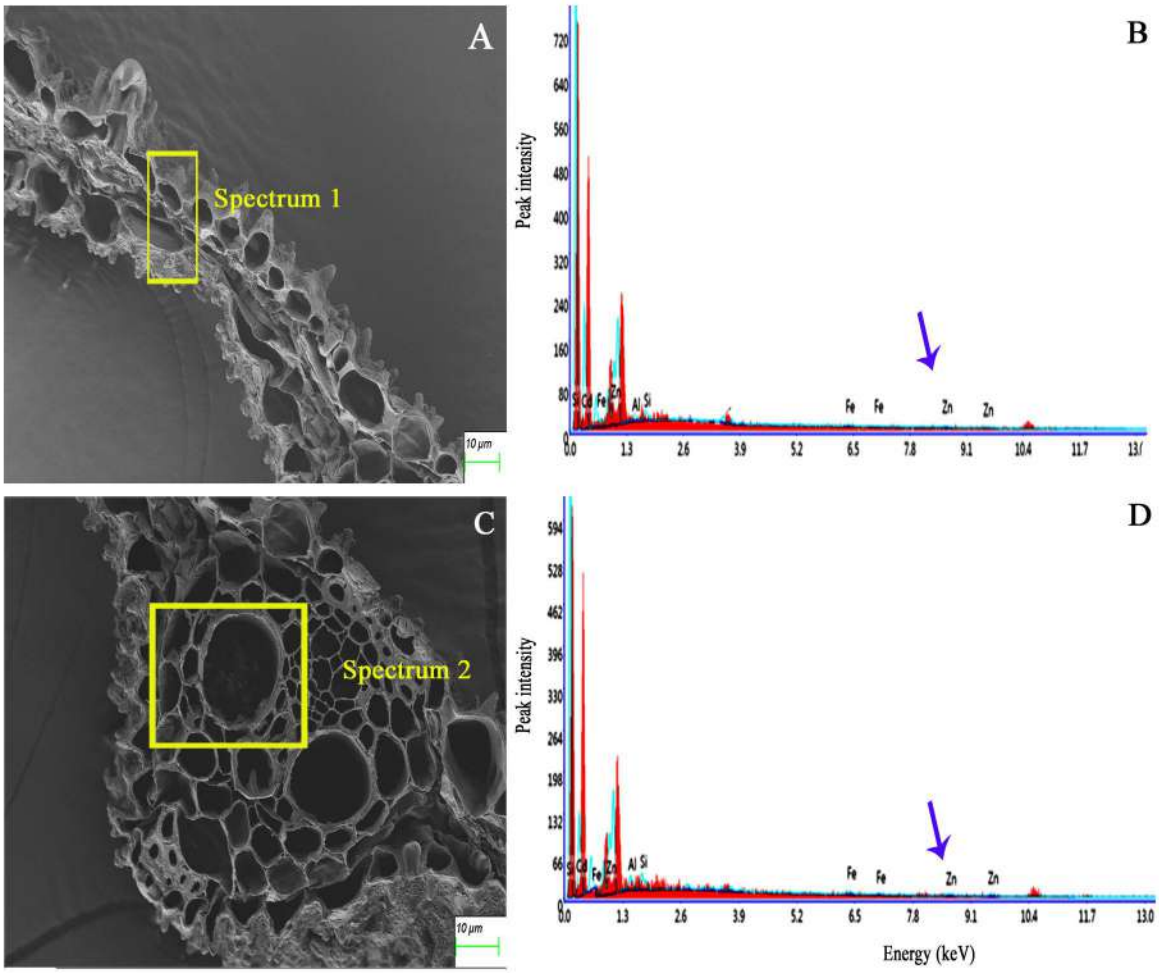

Figure 60: SEM images (A and C) and EDX spectra (B and D) of non-AM plants; two different micro spots were represented as spectrum 1, and spectrum 2 in the leaves of *O. sativa.*

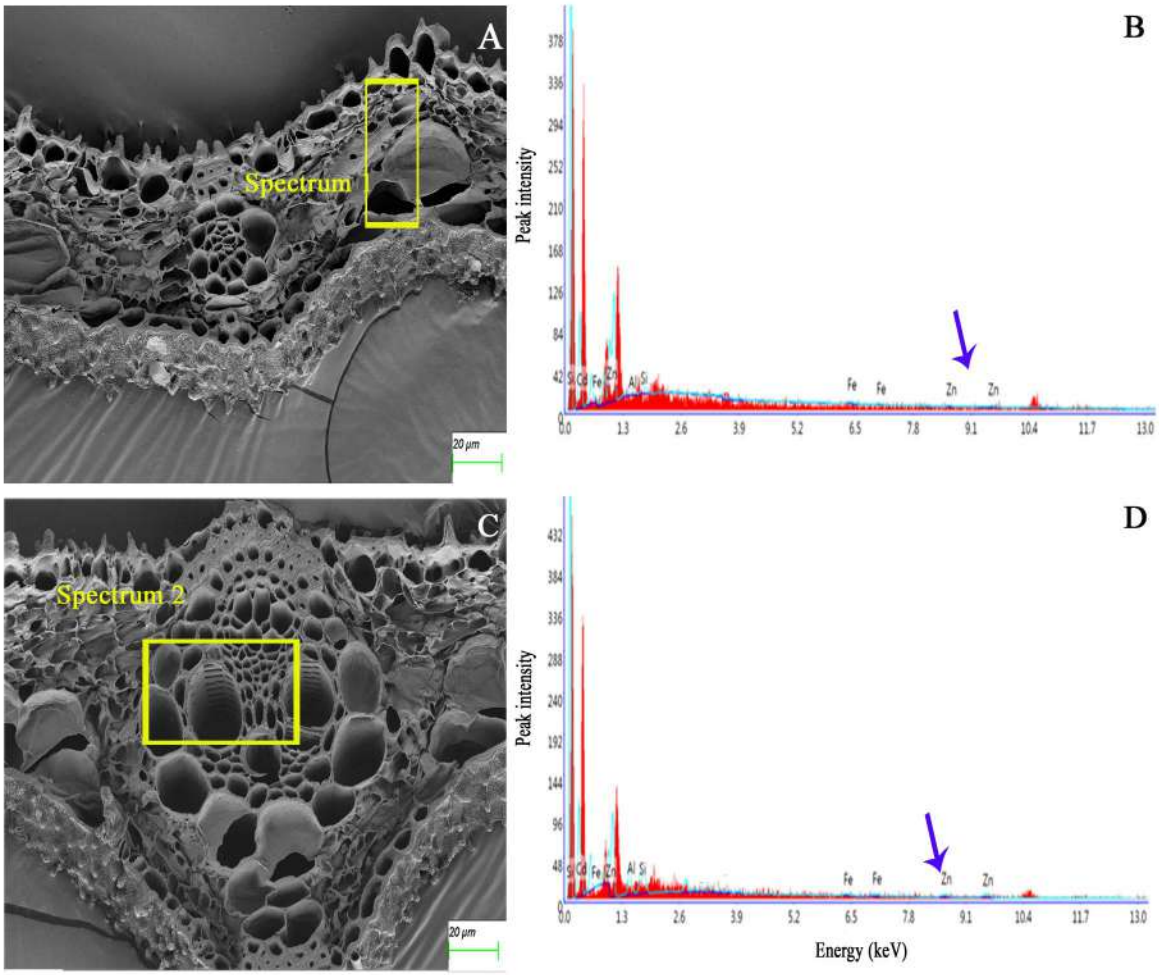

Figure 61: SEM images (A and C) and EDX spectra (B and D) of AM plants; two different micro spots were represented as spectrum 1, and spectrum 2 in the leaves of *O. sativa*.

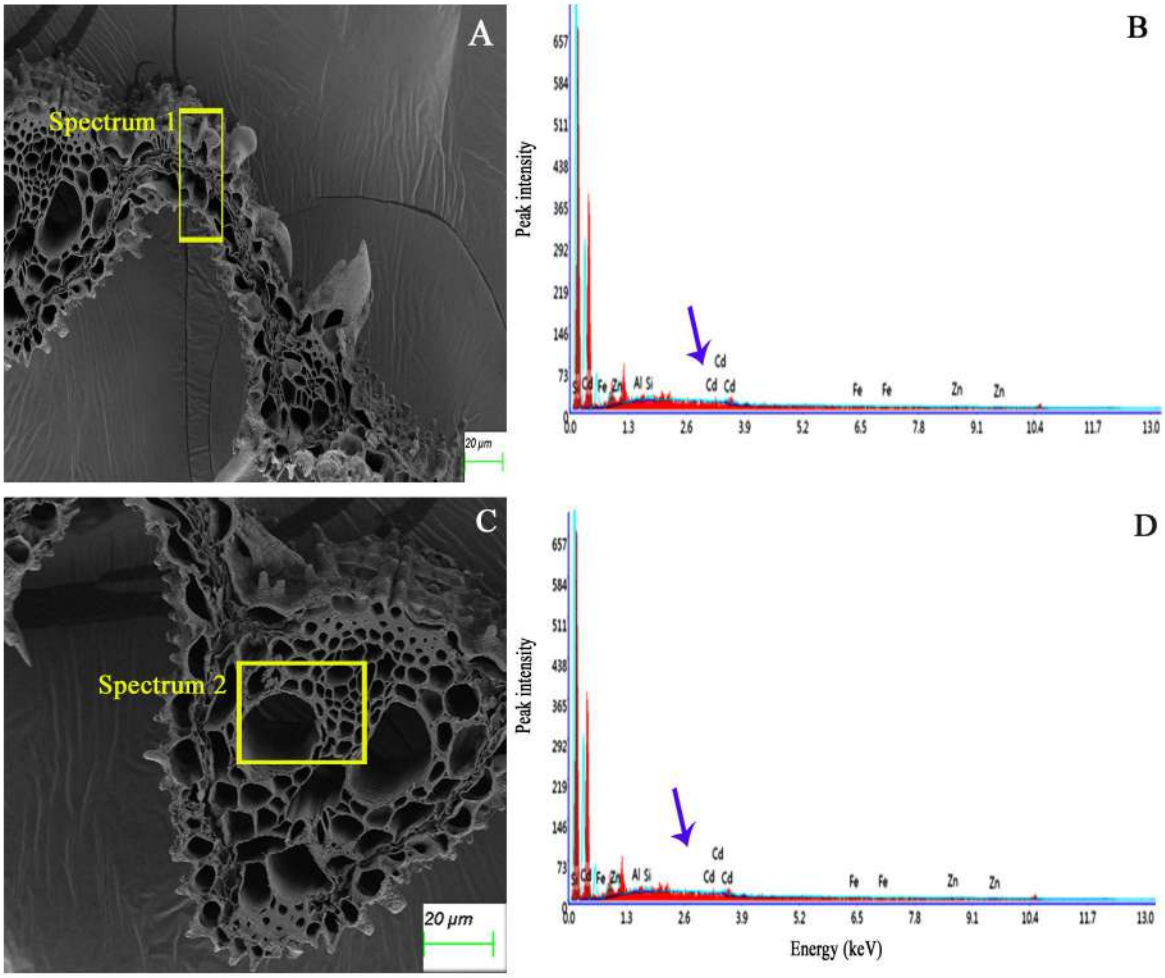

Figure 62: SEM images (A and C) and EDX spectra (B and D) of non-AM plants exposed to Cd stress; two different micro spots were represented as spectrum 1, and spectrum 2 in the leaves of *O. sativa*.

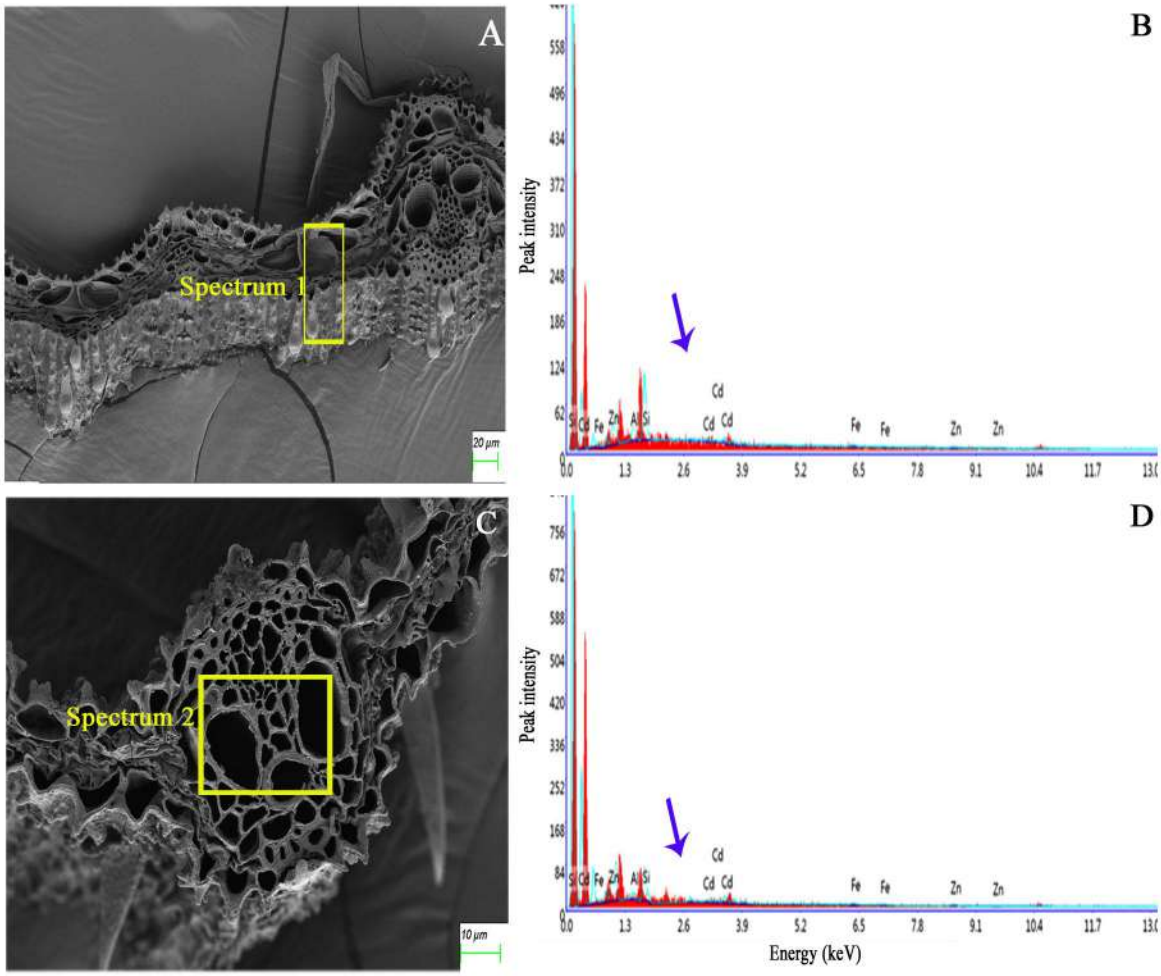

Figure 63: SEM images (A and C) and EDX spectra (B and D) of AM plants exposed to Cd stress; two different micro spots were represented as spectrum 1, and spectrum 2 in the leaves of *O. sativa*.

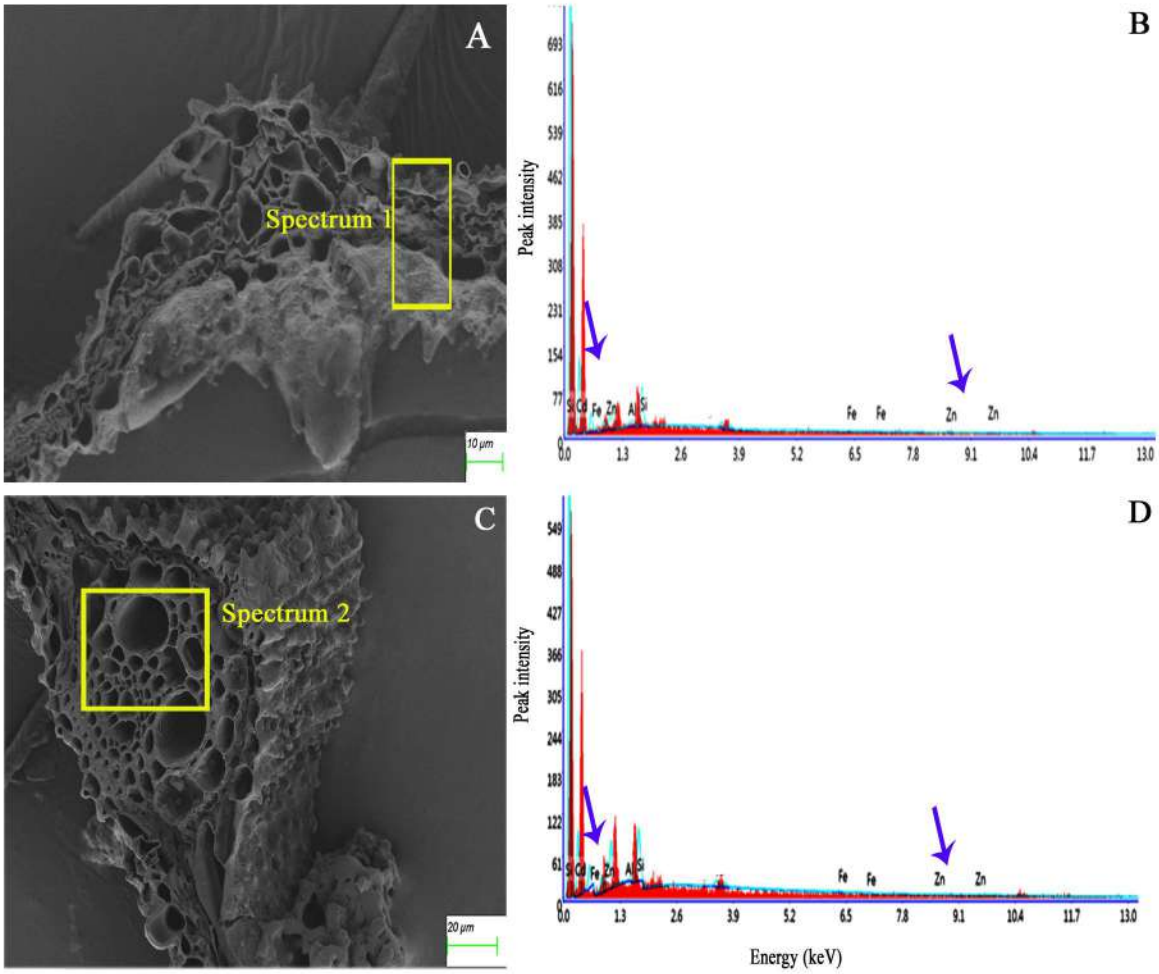

Figure 64: SEM images (A and C) and EDX spectra (B and D) of non-AM plants exposed to Zn stress; two different micro spots were represented as spectrum 1, and spectrum 2 in the leaves of *O. sativa*.

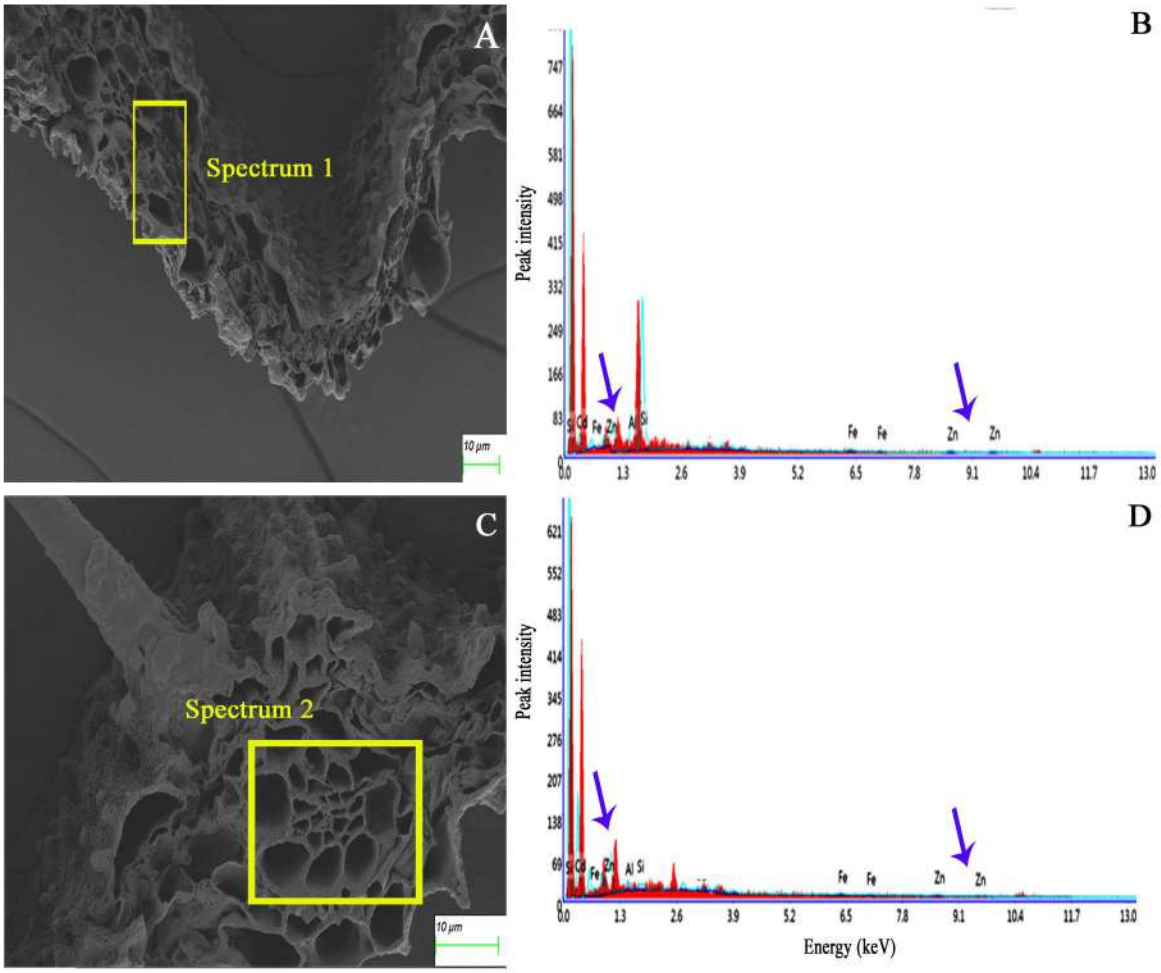

Figure 65: SEM images (A and C) and EDX spectra (B and D) of AM plants exposed to Zn stress; two different micro spots were represented as spectrum 1, and spectrum 2 in the leaves of *O. sativa*.

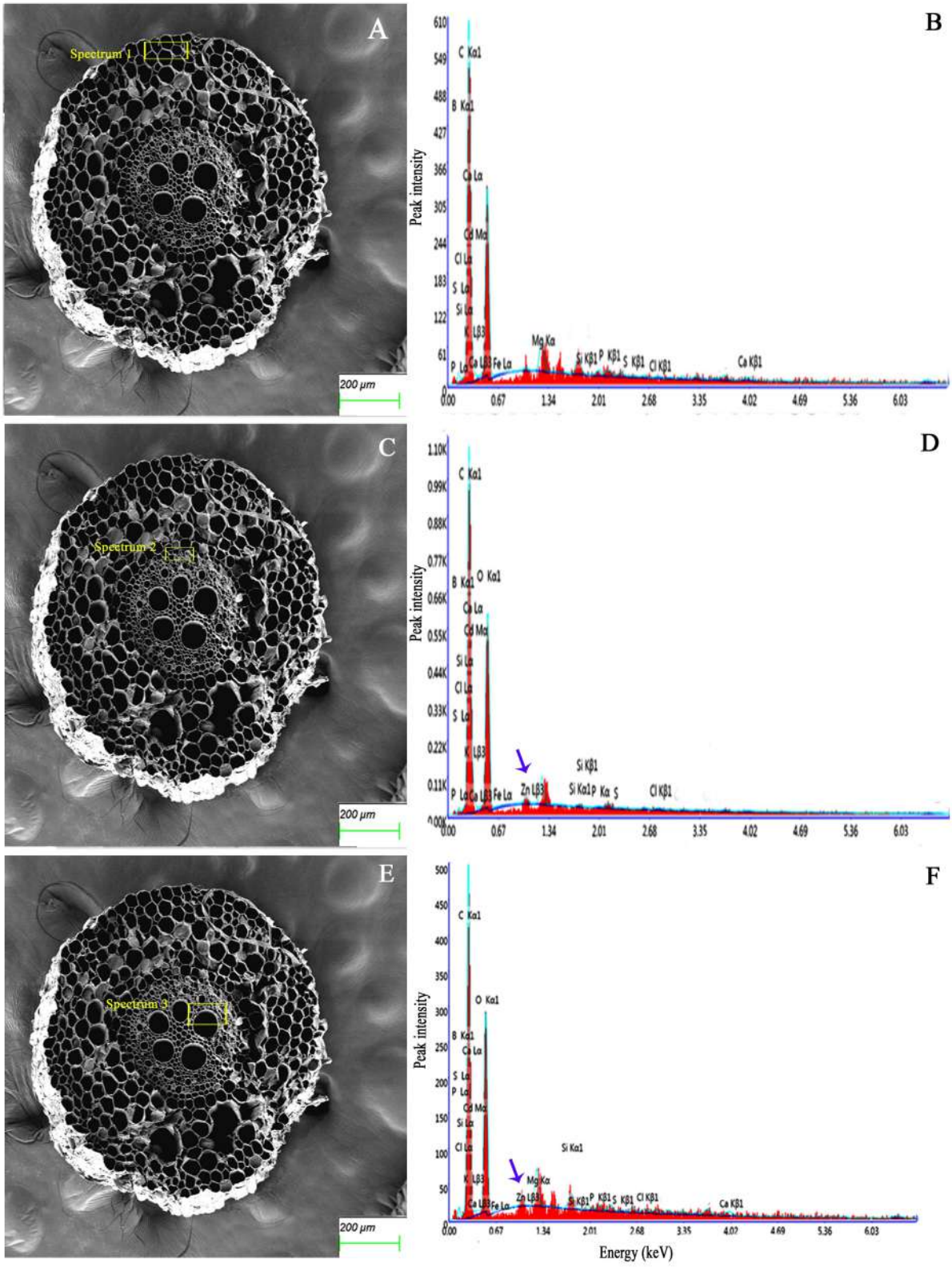

Figure 66: SEM images (A, C and E) and EDX spectra (B, D and E) of non-AM plants; three different micro spots were represented as spectrum 1, spectrum 2 and spectrum 3, in roots of *Z. mays*.

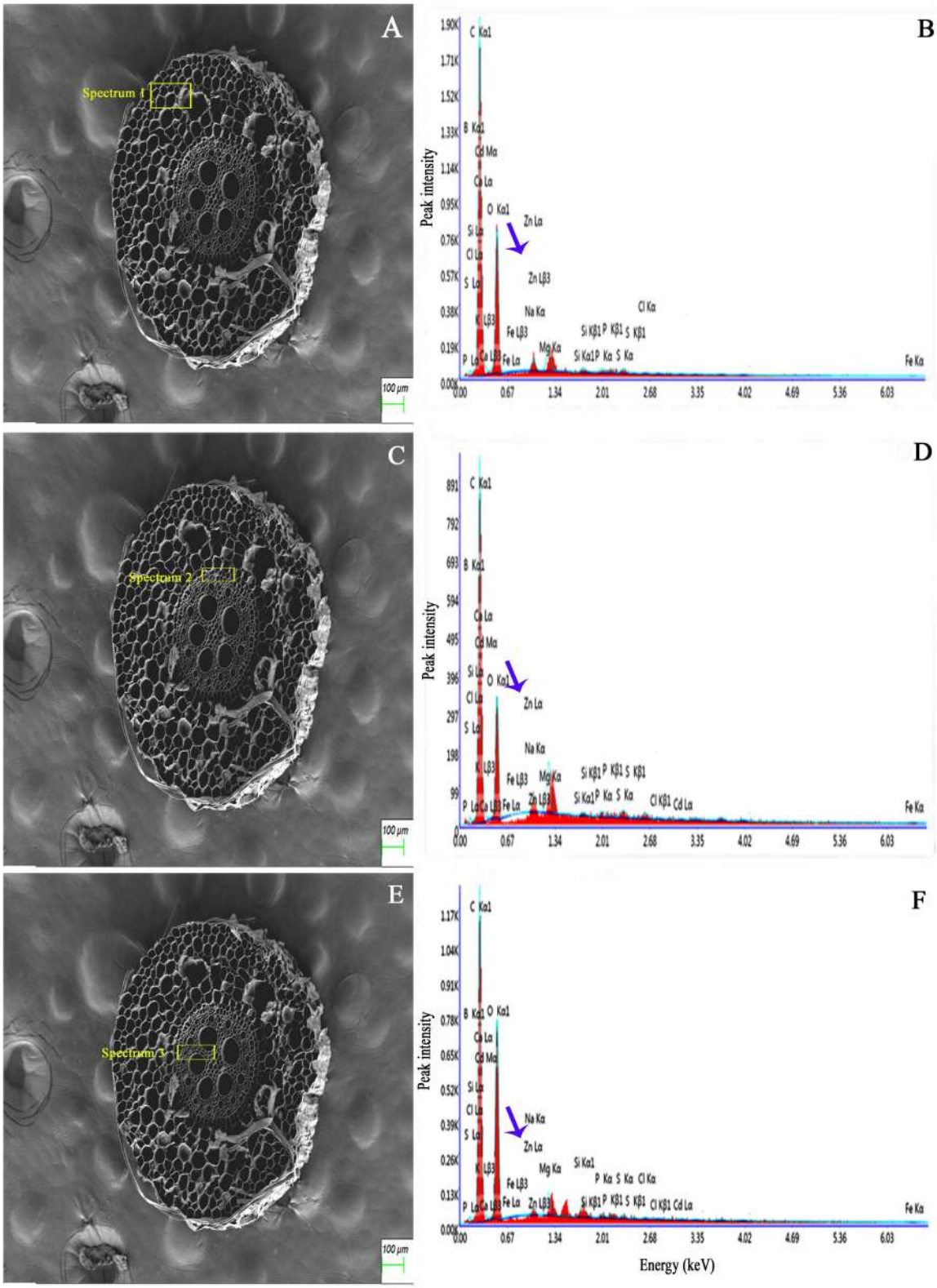

Figure 67: SEM images (A, C and E) and EDX spectra (B, D and E) of AM plants; three different micro spots were represented as spectrum 1, spectrum 2 and spectrum 3, in roots of *Z. mays*.

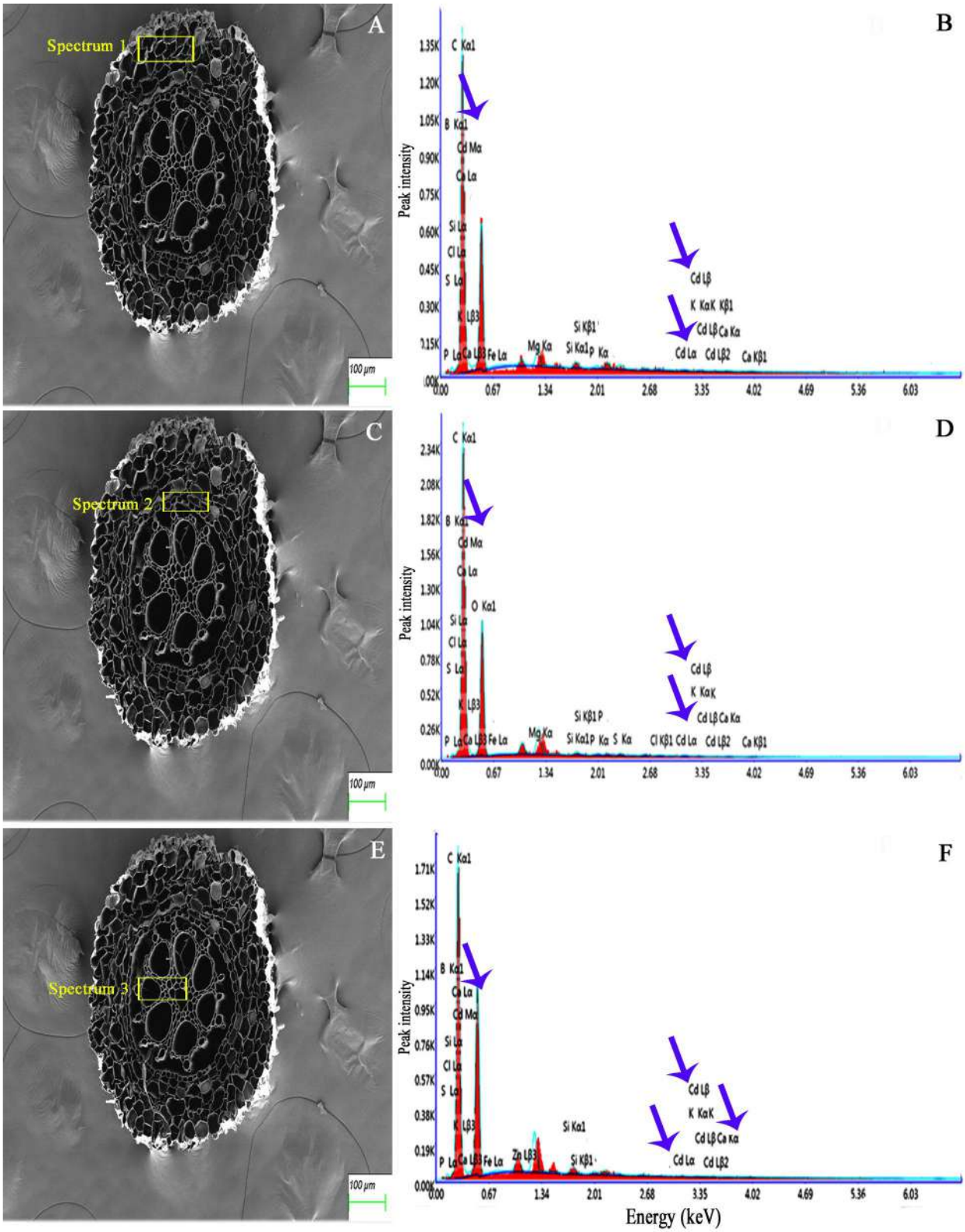

Figure 68: SEM images (A, C and E) and EDX spectra (B, D and E) of non-AM plants exposed to Cd stress; three different micro spots were represented as spectrum 1, spectrum 2 and spectrum 3, in roots of *Z. mays*.

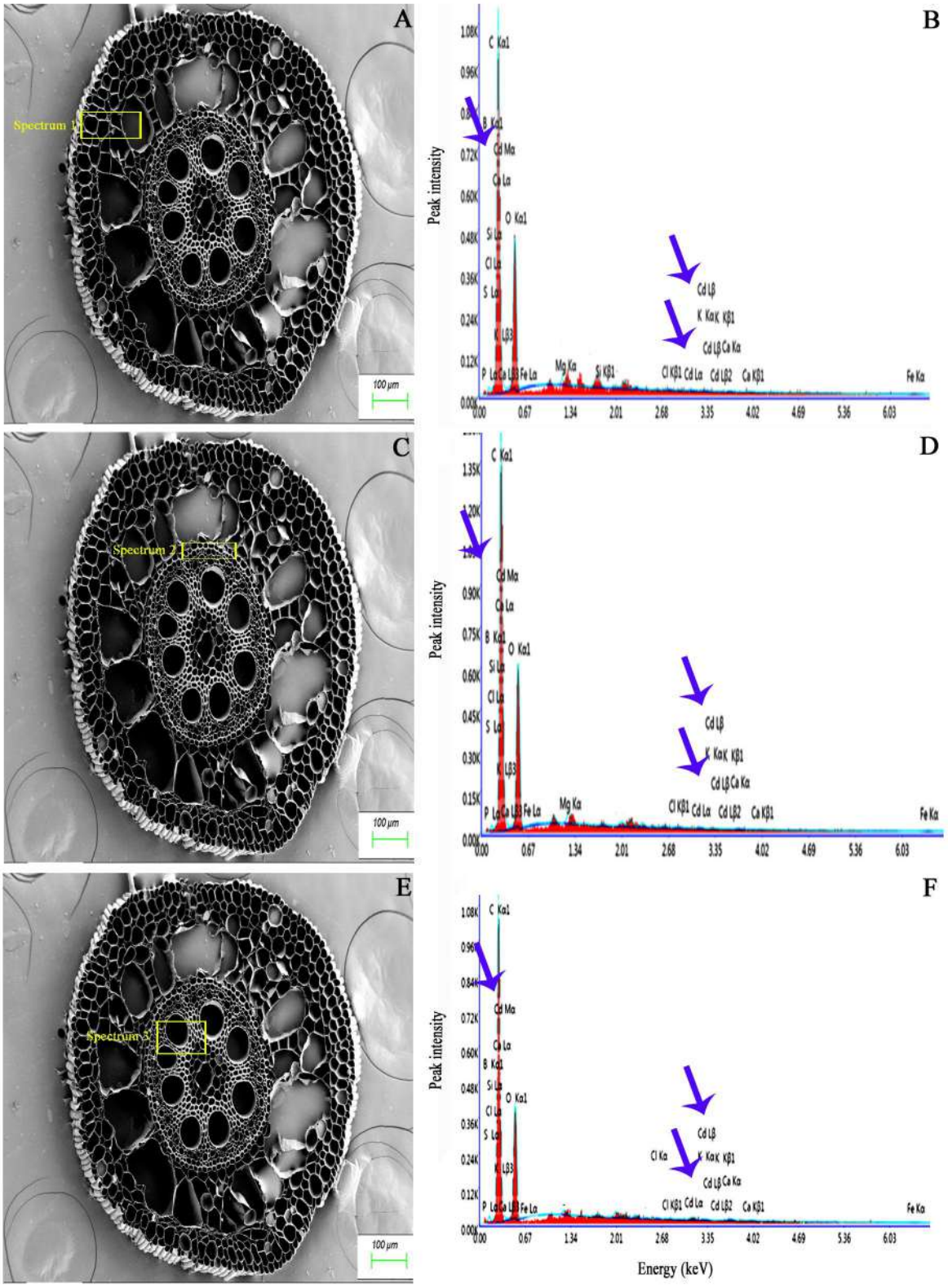

Figure 69: SEM images (A, C and E) and EDX spectra (B, D and E) of AM plants exposed to Cd stress; three different micro spots were represented as spectrum 1, spectrum 2 and spectrum 3, in roots of *Z. mays*.

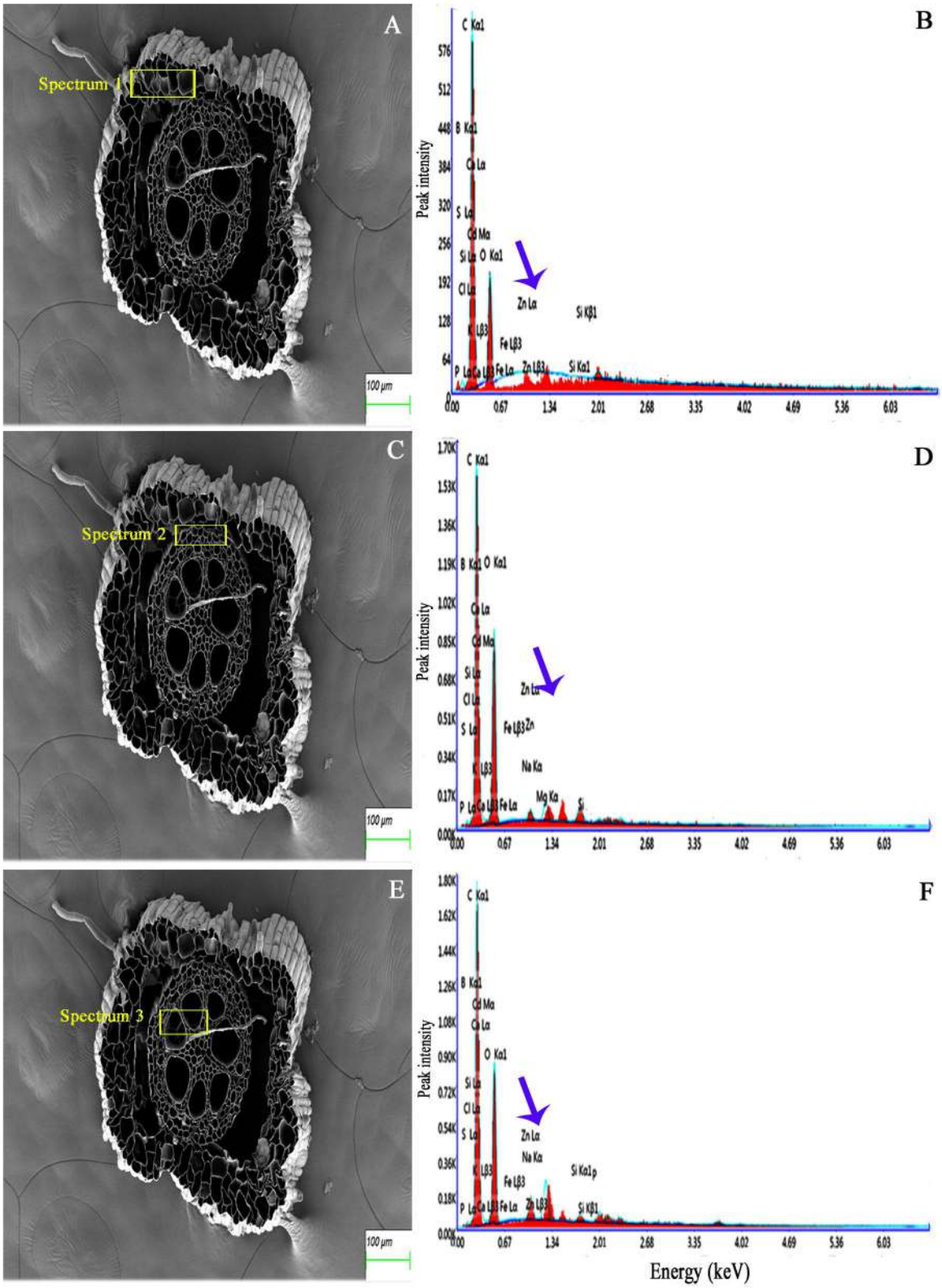

Figure 70: SEM images (A, C and E) and EDX spectra (B, D and E) of non-AM plants exposed to Zn stress; three different micro spots were represented as spectrum 1, spectrum 2 and spectrum 3, in roots of *Z. mays*.

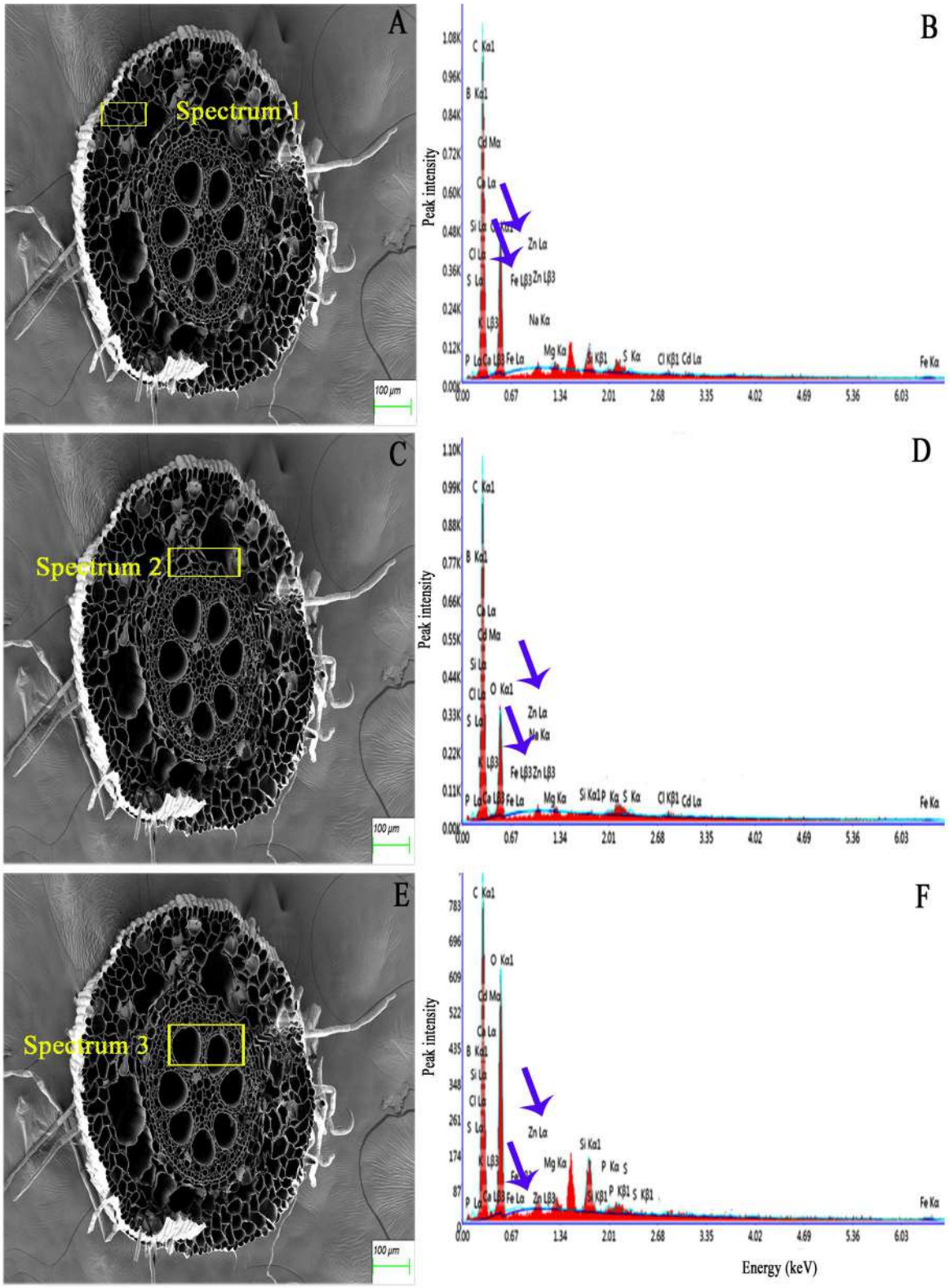

Figure 71: SEM images (A, C and E) and EDX spectra (B, D and E) of AM plants exposed to Zn stress; three different micro spots were represented as spectrum 1, spectrum 2 and spectrum 3, in roots of *Z. mays*.

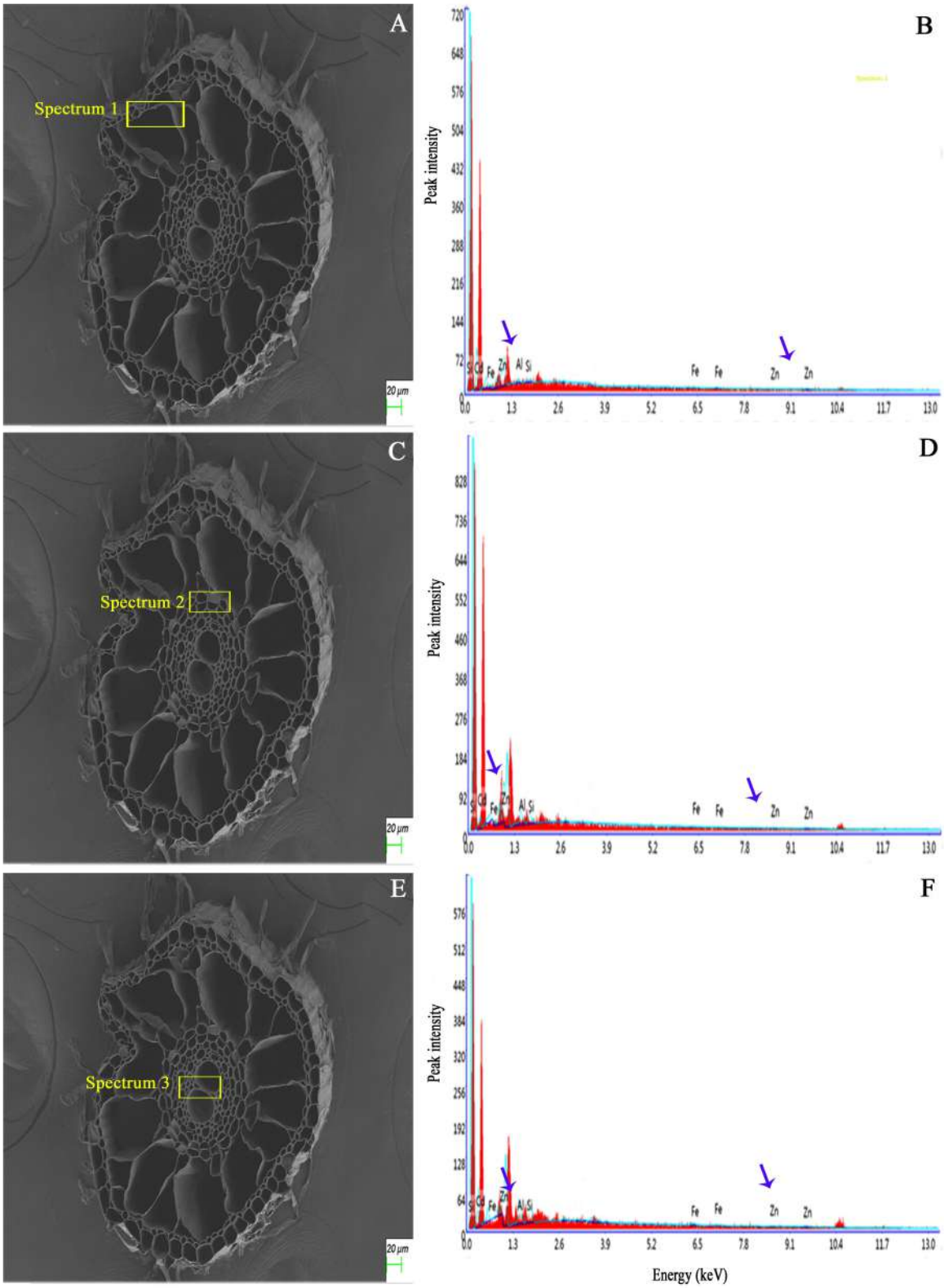

Figure 72: SEM images (A, C and E) and EDX spectra (B, D and E) of non-AM plants; three different micro spots were represented as spectrum 1, spectrum 2 and spectrum 3, in roots of *O. sativa.*

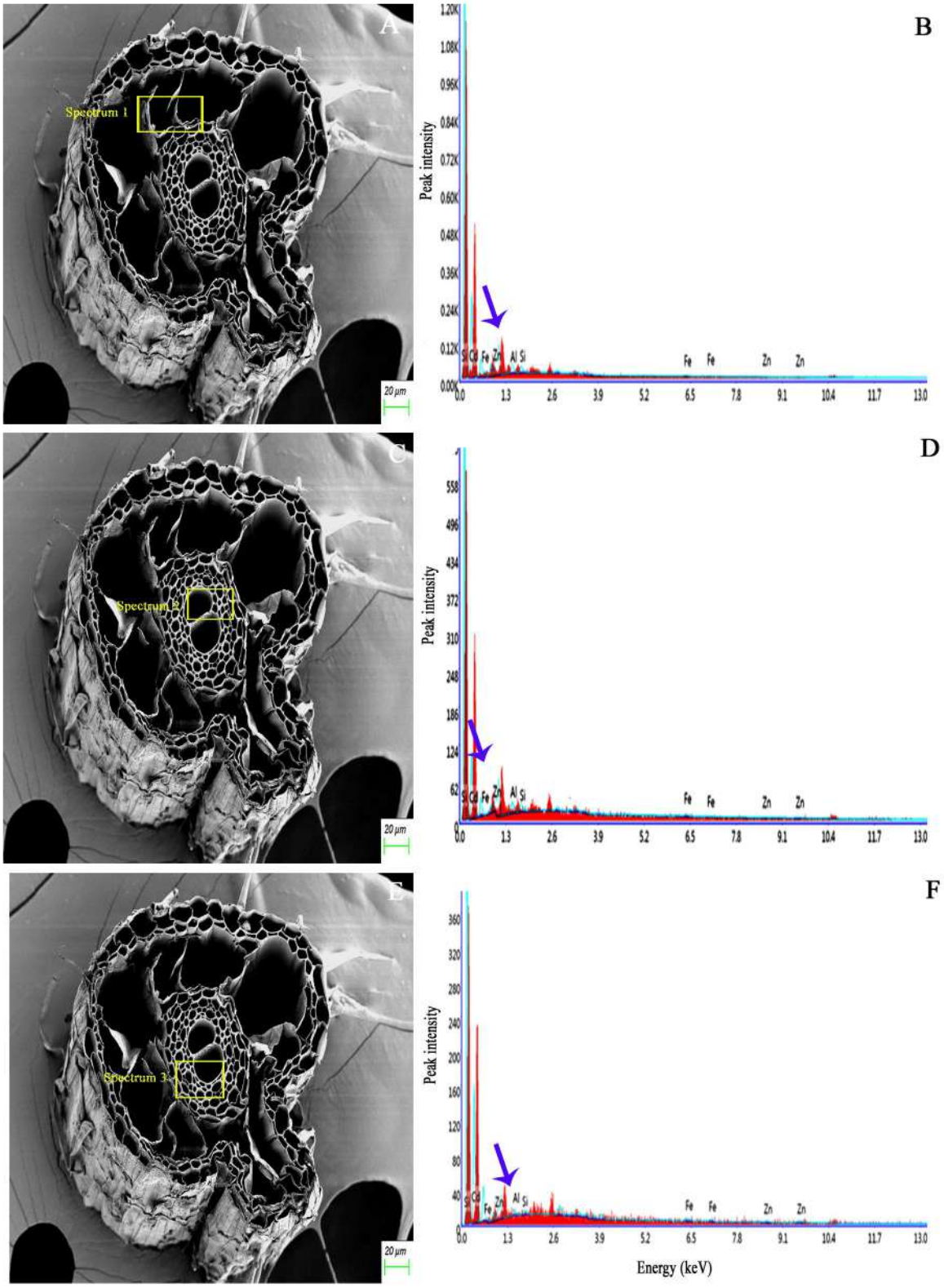

Figure 73: SEM images (A, C and E) and EDX spectra (B, D and E) of AM plants exposed to Cd; three different micro spots were represented as spectrum 1, spectrum 2 and spectrum 3, in roots of *O. sativa*.

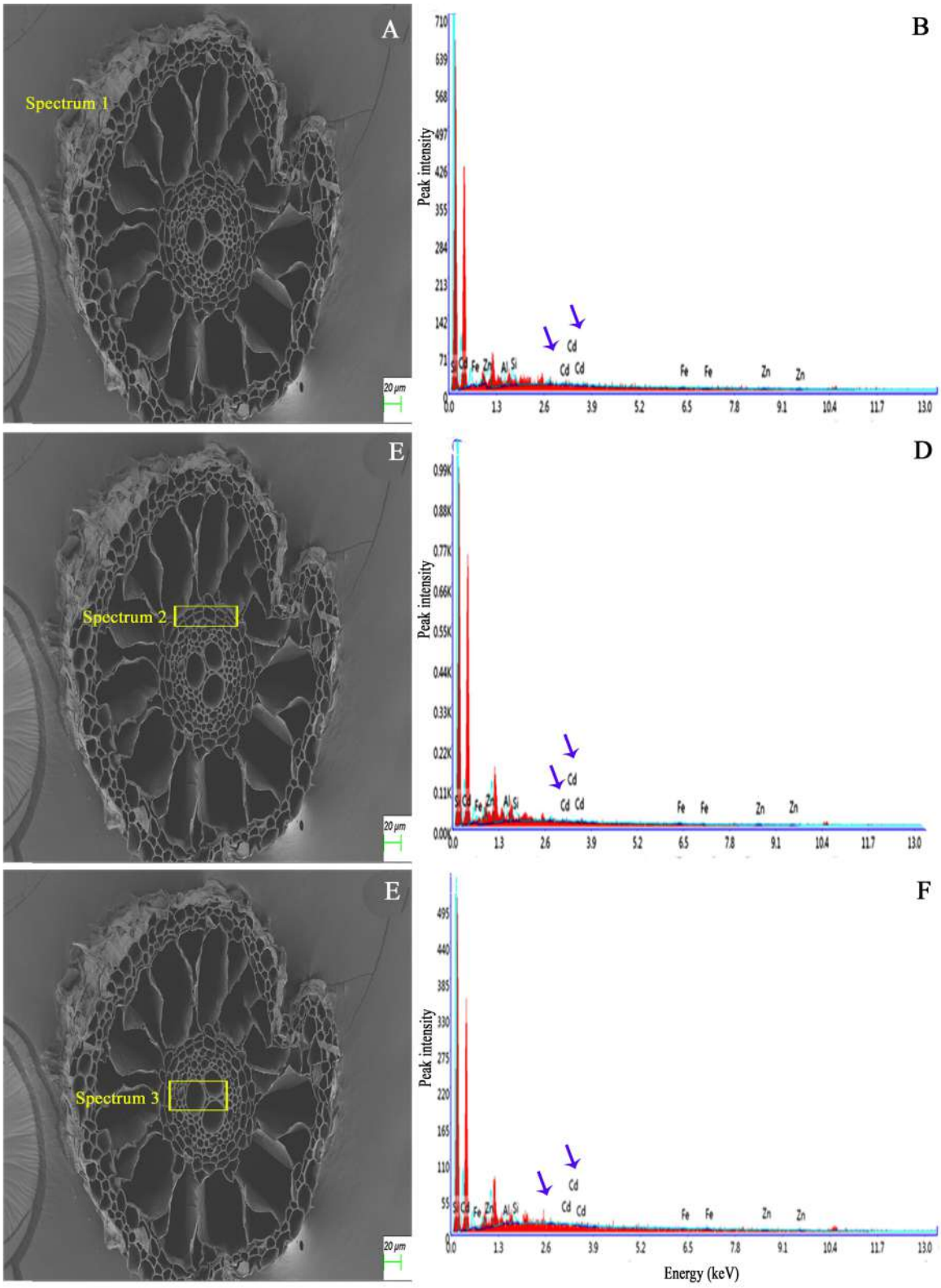

Figure 74: SEM images (A, C and E) and EDX spectra (B, D and E) of non-AM plants exposed to Cd stress; three different micro spots were represented as spectrum 1, spectrum 2 and spectrum 3, in roots of *O. sativa*.

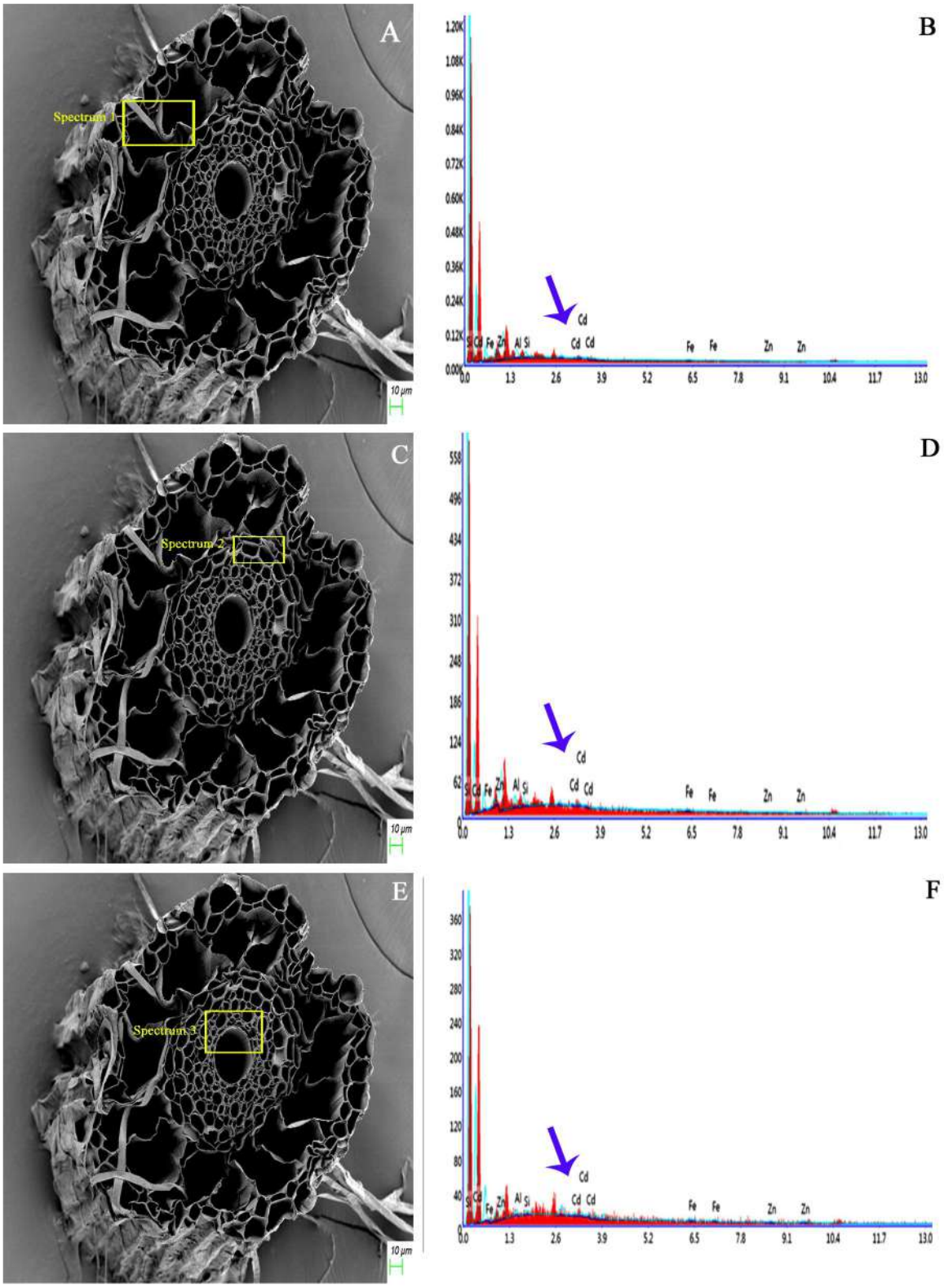

Figure 75: SEM images (A, C and E) and EDX spectra(B, D and E) of AM plants exposed to Cd stress; three different micro spots were represented as spectrum 1, spectrum 2 and spectrum 3, in roots of *O. sativa*.

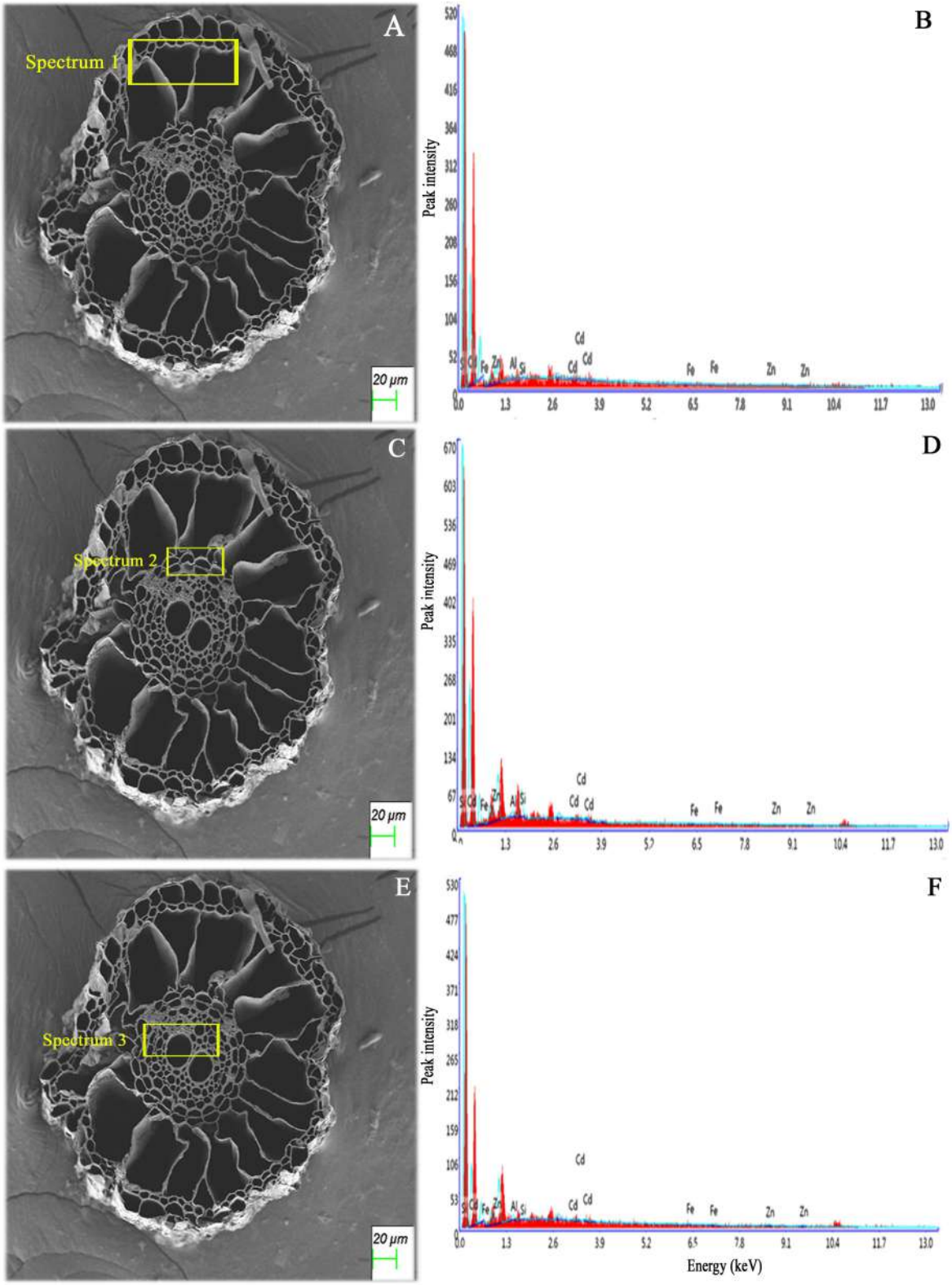

Figure 76: SEM images (A, C and E) and EDX spectra (B, D and E) of non-AM plants exposed to Zn stress; three different micro spots were represented as spectrum 1, spectrum 2 and spectrum 3, in roots of *O. sativa*.

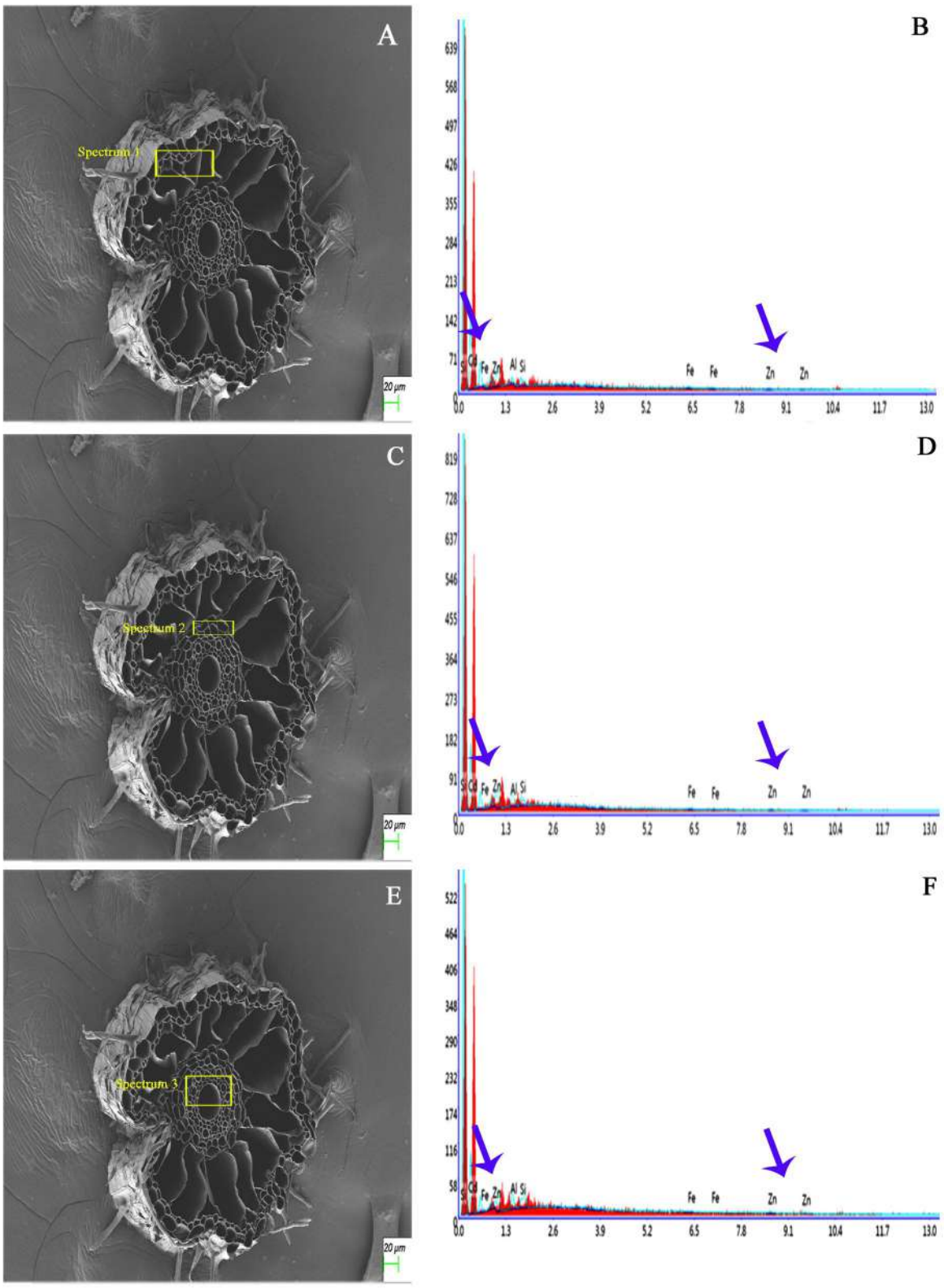

Figure 77: SEM images (A, C and E) and EDX spectra (B, D and E) of AM plants exposed to Zn stress; three different micro spots were represented as spectrum 1, spectrum 2 and spectrum 3, in roots of *O. sativa.*

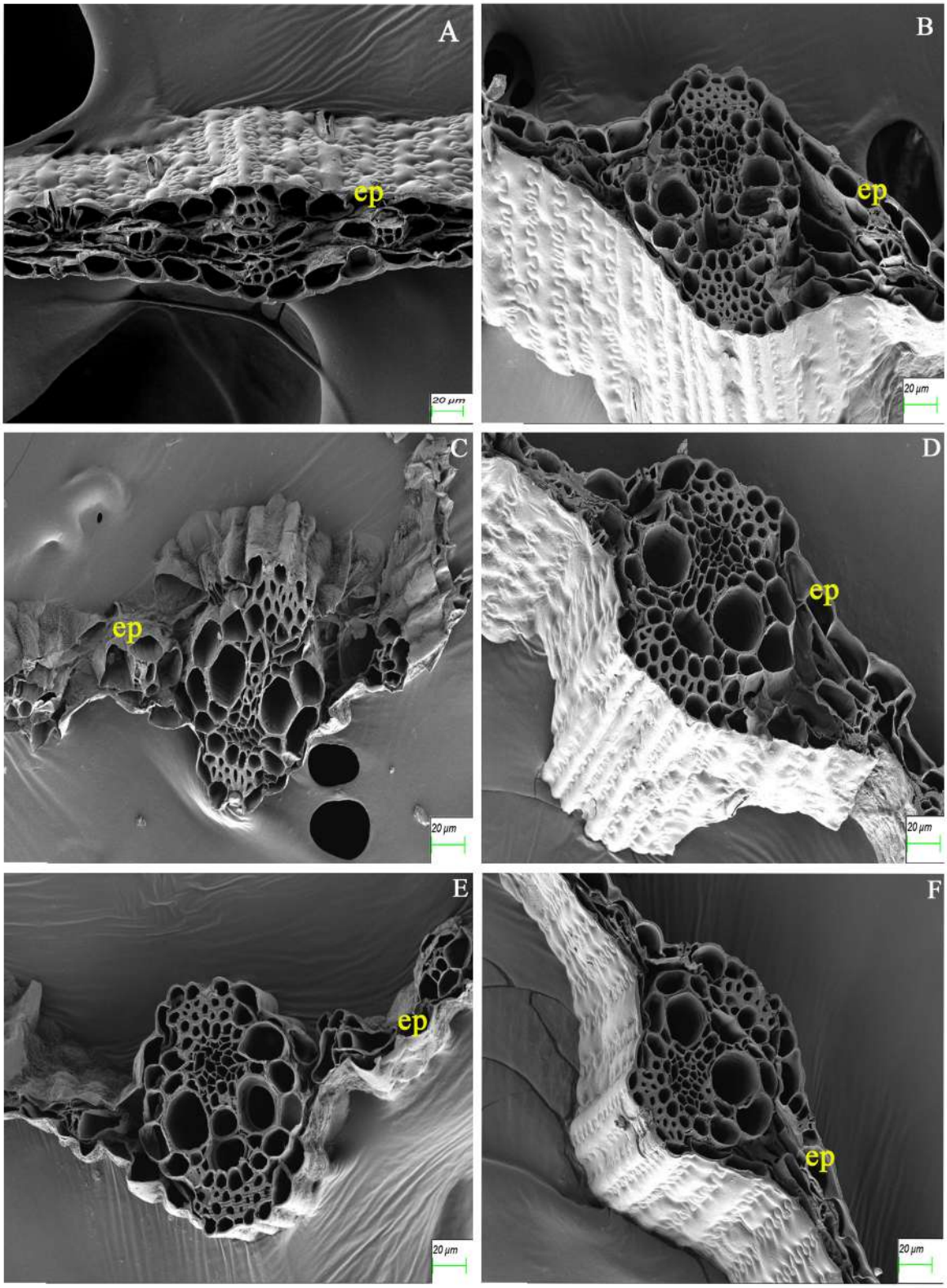

Figure 78: Scanning electron micrographs of leaf cross sections of *Z. mays* plants associated with mycorrhiza exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd, D) AM+Cd, E) non-AM+Zn and F) AM+Zn; where ep-epidermis.

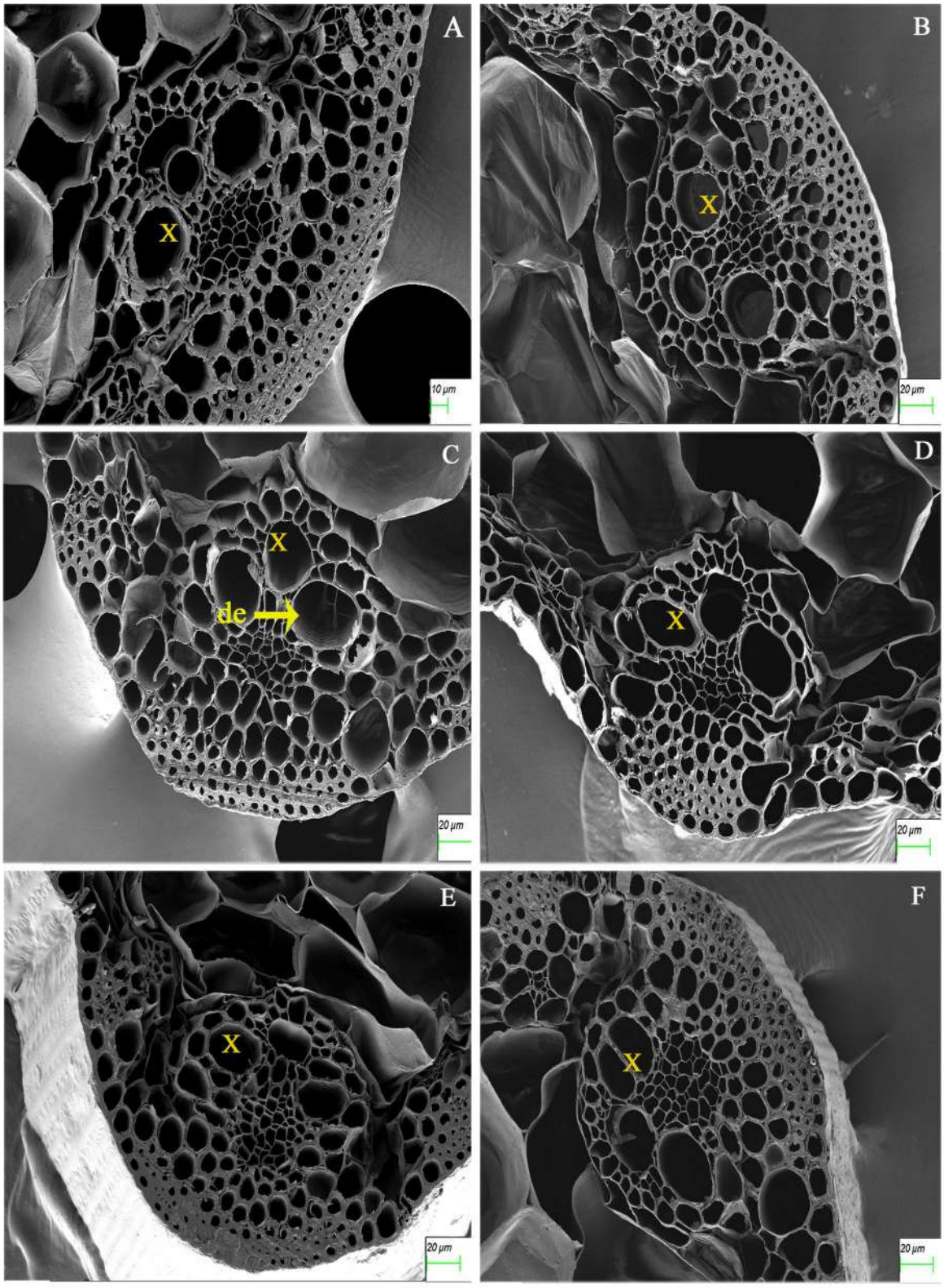

Figure 79: Scanning electron micrographs of leaf stelar regions of *Z. mays* plants associated with mycorrhiza exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd, D) AM+Cd, E) non-AM+Zn and F) AM+Zn; where X- xylem, de- depositions.

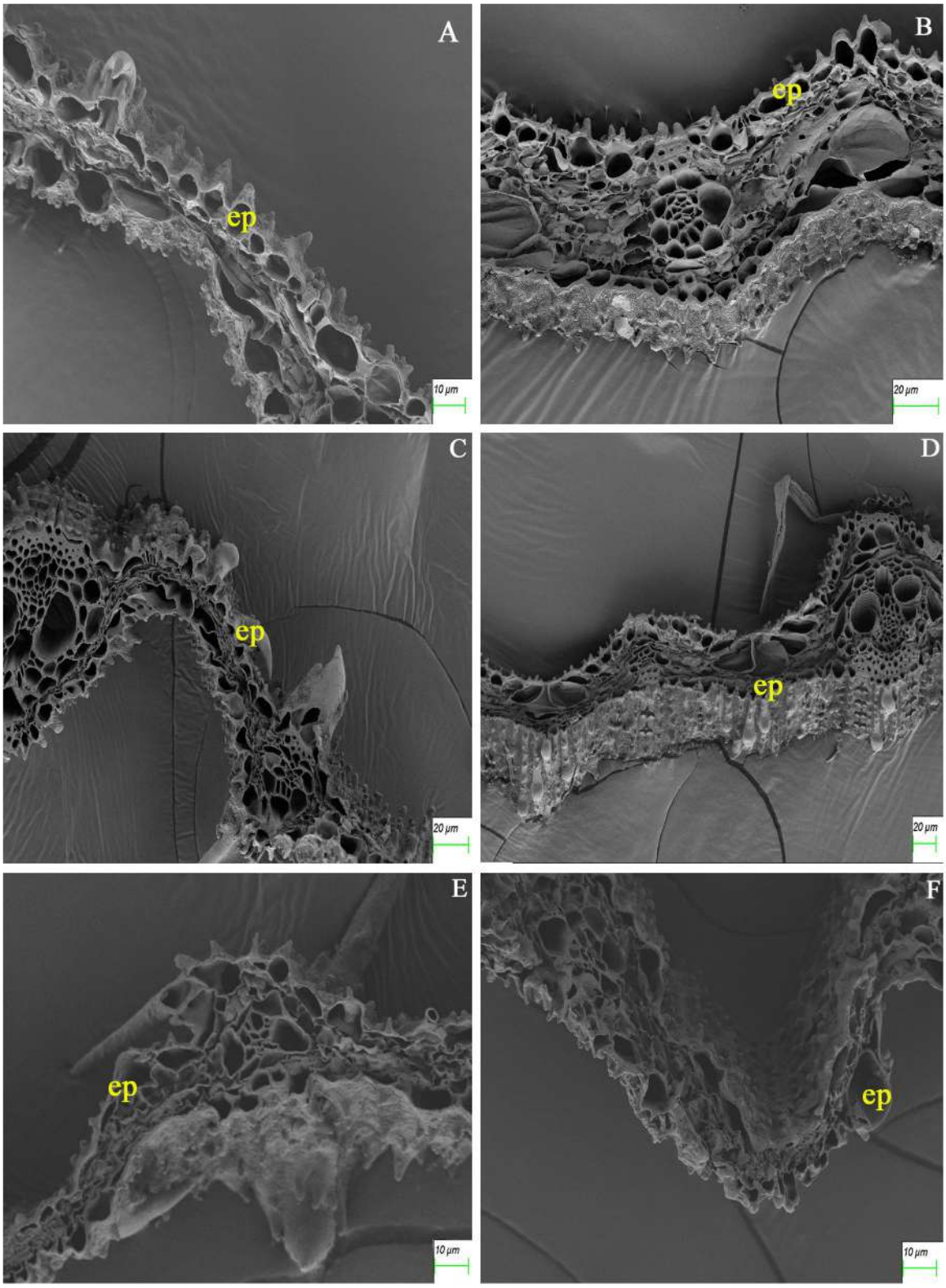

Figure 80: Scanning electron micrographs of leaf cross sections of *O. sativa* plants associated with mycorrhiza exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd, D) AM+Cd, E) non-AM+Zn and F) AM+Zn; where ep-epidermis.

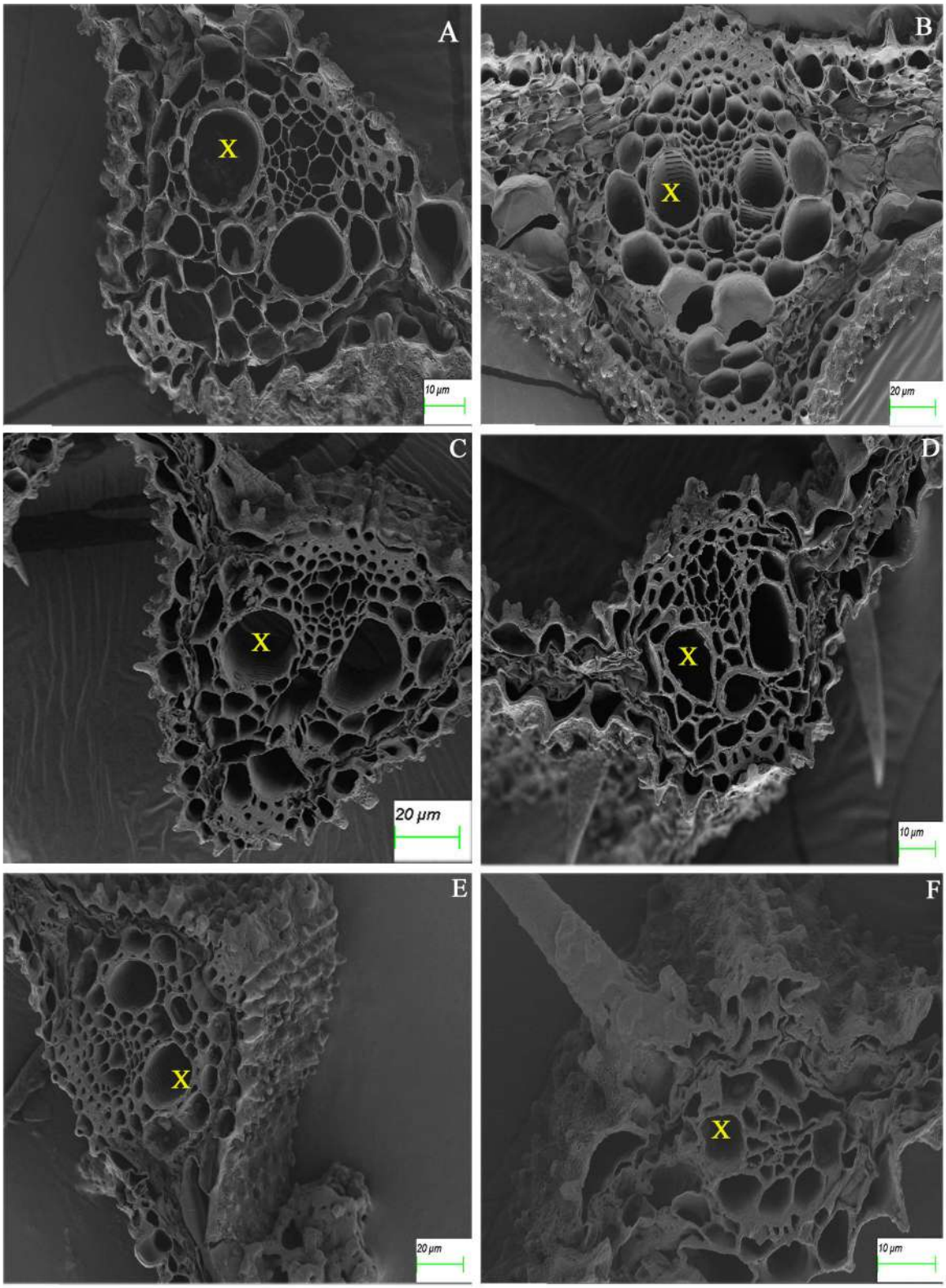

Figure 81: Scanning electron micrographs of leaf stelar regions of *O. sativa* plants associated with mycorrhiza exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd, D) AM+Cd, E) non-AM+Zn and F) AM+Zn; where X-xylem.

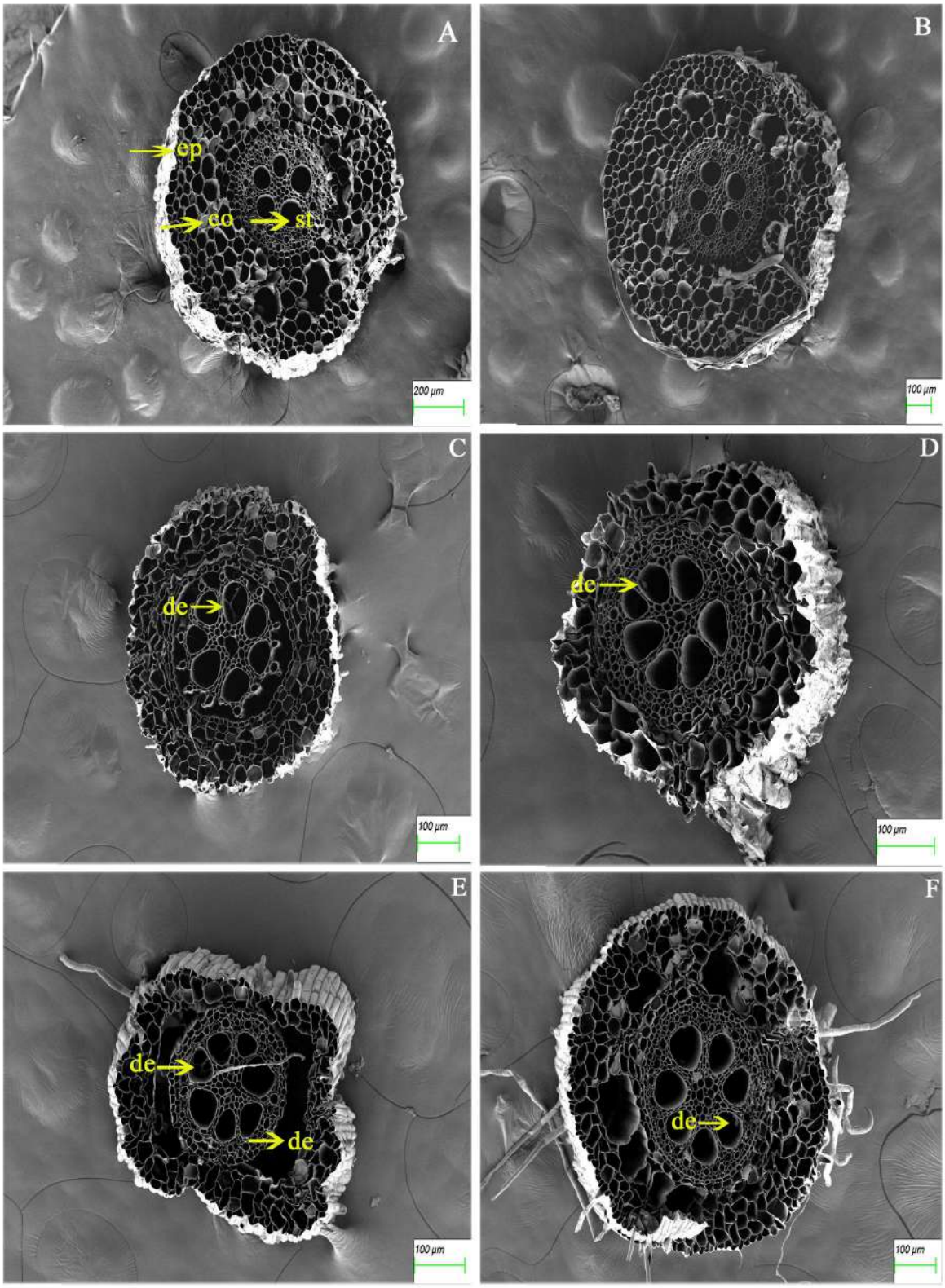

Figure 82: Scanning electron micrographs of root cross sections of *Z. mays* plants associated with mycorrhiza exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd, D) AM+Cd, E) non-AM+Zn and F) AM+Zn; where ep-epidermis, c-cortex, de-depositions.

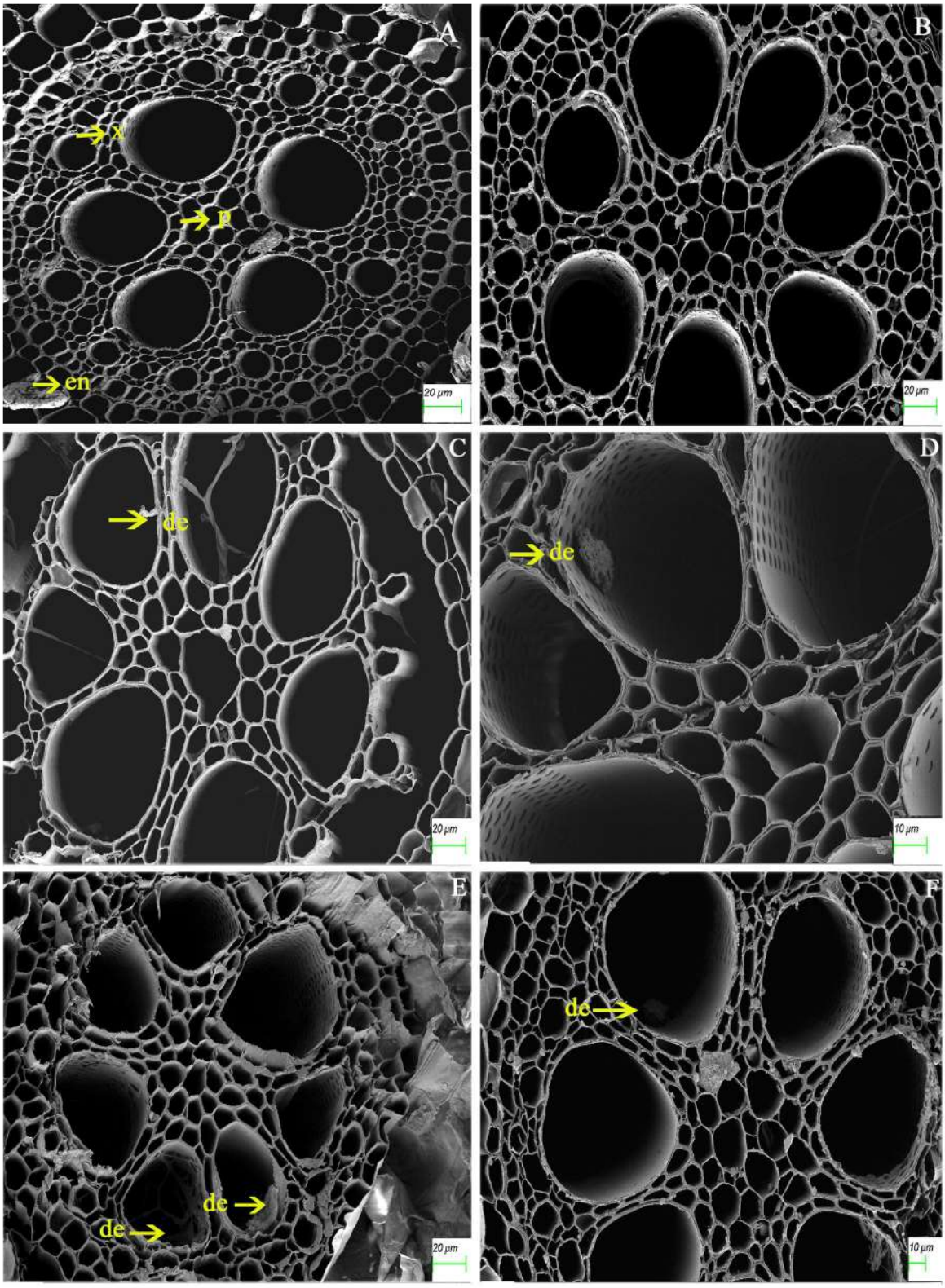

Figure 83: Scanning electron micrographs of root stelar regions of *Z. mays* plants associated with mycorrhiza exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd, D) AM+Cd, E) non-AM+Zn and F) AM+Zn; where en-endodermis, c-cortex, p-pith, x-xylem, de-depositions.

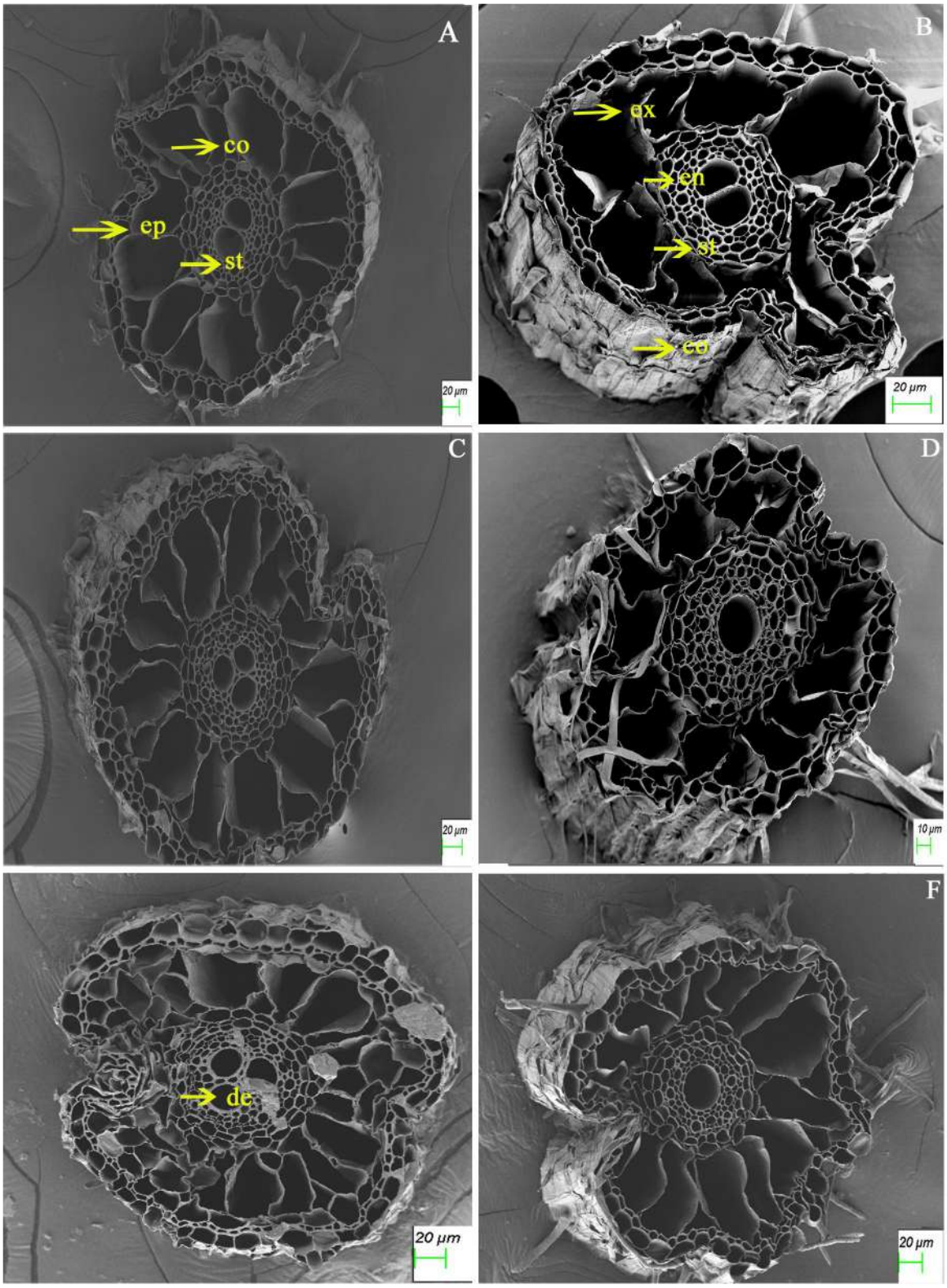

Figure 84: Scanning electron micrographs of root cross sections of *O. sativa* plants associated with mycorrhiza exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd, D) AM+Cd, E) non-AM+Zn and F) AM+Zn; where ep-epidermis, ex-exodermis, en-endodermis, c-cortex, de-depositions.

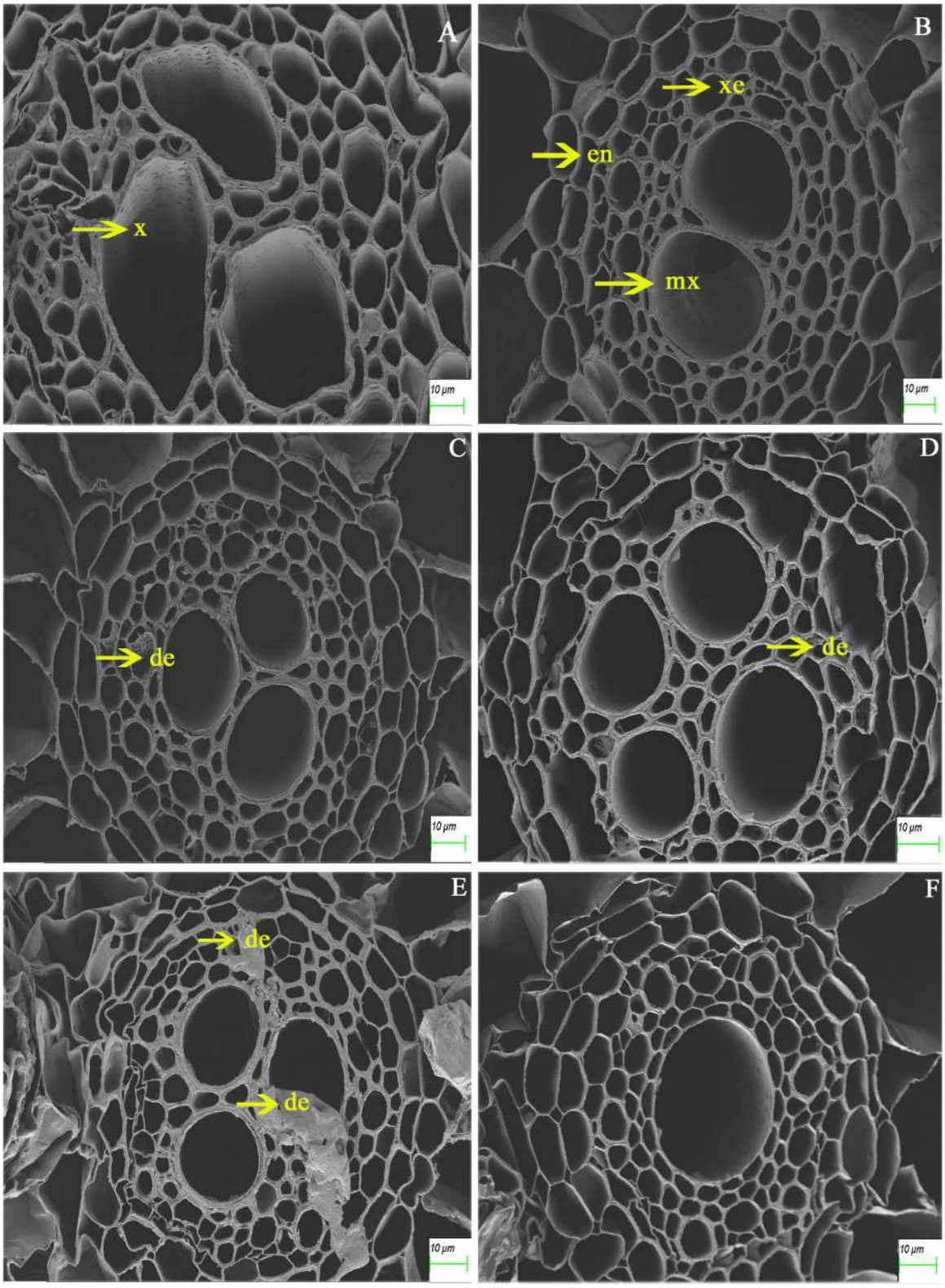

Figure 85: Scanning electron micrographs of root stelar regions of *O. sativa* associated with mycorrhiza exposed to Cd (0.45 g kg^{-1}) and Zn (1. 95 g kg^{-1}) stresses; A) non-AM, B) AM, C) non-AM+Cd, D) AM+Cd, E) non-AM+Zn and F) AM+Zn; where ep-epidermis, c-cortex, de-depositions.

Table 14: Bioactive compounds detected in leaves of non-AM and AM plants of *Z. mays* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses.

Bioactive compounds	Area percentage (%)					
	Non-AM	AM	Non-AM+Cd	AM+Cd	Non-AM+Zn	AM+Zn
1,2-benzenedicarboxylic acid, diisooctyl ester	-	1.87	-	-	-	-
3beta-acetoxystigmasta-4,6,22-triene	-	-	3.35	-	-	-
Beta-linalool	-	-	-	2.47	-	-
Cholesta-4,6-dien-3-ol, benzoate, (3beta)-	-	2.34	5.48	2.18	3.31	3.86
Gamma-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

Table 15: Bioactive compounds detected in leaves of non-AM and AM plants of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses.

Bioactive compounds	Area percentage (%)					
	Non-AM	AM	Non-AM+Cd	AM+Cd	Non-AM+Zn	AM+Zn
Neophytadiene	44.5	40.88	18.27	20.68	30.81	31.32
(22e)-stigmasta-4,6,22-trien-3-yl acetate	-	-	-	-	1.44	-
(E)-phytol	10.9	11.15	-	7.85	13.95	10.26
1,2-benzenedicarboxylic acid, diisooctyl ester	3.47	5.38	2.75	-	-	-
22-dihydroergosterol	-	-	-	3.06	-	-
2-Hexadecen-1-ol, 3,7,11,15-tetramethyl	-	-	7.74	-	-	16.11
2pentadecanon, 6,10,14-trimethyl-	-	-	-	-	4.14	4.42
3,6-octadecadienoic acid, methyl ester	3.2	-	-	-	0	0
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	7.28	7.46	5.43	9.28	8.84	16.11
3.beta.-acetoxystigmasta-4,6,22-triene	-	-	-	-	-	2.93
9,12- octadecadienoic acid (z,z)-, methyl ester	-	-	-	-	1.59	-
Cholesta-4,6-dien-3-ol, benzoate, (3.beta.)-	-	-	2.55	2.09	-	2.15
Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	-	-	-	-	2.73	-
Dihydroergosterol			3.22	-	-	-
Ethyl iso-allocholate	1.73	1.37	-	-	-	-
Hexadecadienoic acid, methyl ester	-	2.75	-	-	3.73	-
Hexadecanoic acid	-	-	5.81	-	-	-
Linoleic acid, methyl ester	-	-	-	-	-	1.41
Methyl isoheptadecanoate	-	3.33	-	-	-	-
Methyl linolenate	-	-	-	-	-	3.17
Methyl palmitate	3.75	-	-	-	-	5.38
Methyl stearate	-	-	-	-	-	0.88
Phytol	25.17	27.69	52.38	56.13	3.68	21.83
Squalene			1.85	0.91	1.74	-

Table 16: Phytol composition in the leaves of non-AM and AM plants of *Z. mays* and *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses.

Treatments	*Z. mays*			*O. sativa*		
	Area percentage (%)					
	Phytol	(E)-phytol	Phytol /(E)-phytol	Phytol	(E)-phytol	Phytol /(E)-phytol
Non-AM	26.96	11.81	2.28	25.17	10.9	2.309174
AM	17.06	9.12	1.87	27.69	11.15	2.483408
Non-AM+Cd	23.41	4.91	4.77	52.38	-	-
AM+Cd	31.11	7.68	4.05	56.13	7.85	7.150318
Non-AM+Zn	13.8	16.49	0.84	3.68	13.95	0.263799
AM+Zn	13.72	17.15	0.80	21.83	10.26	2.12768

Table 17: Bioactive compounds detected in roots of non-AM and AM plants of *Z. mays* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses.

Bioactive compounds	Area percentage (%)					
	Non-AM	AM	Non -AM+Cd	AM+Cd	Non-AM+Zn	AM+Zn
Eicosane	2.53	-	-	12.07	-	1.97
Gamma.-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-beta-hydroxydigitoxigenin	-	-	4.56			
1-heneicosanol	3.25	-	-	1.73	2.65	6.27
1-hexadecanol	-	-	-	-	-	2.75
2-pentadecanone, 6,10,14-trimethyl-	-	-	-	-	1.37	-
3beta-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, (3.beta.)-	-	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, (3beta)-	2.64	-	1.68	-	-	-
Cycloartenol		-	-	-	1.59	-
Diethylene glycol dibenzoate	2.54	-	-	-	2.12	-
Eicosanoic acid, methyl ester	-	-	-	-	6.27	-
Elixene	-	-	2.22	-		-
Glycerol beta-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	-	-	-	-	-
Nonadecane	-	-	-	-	1.81	2.13
Octadecane	-	1.42	-	3.61	-	-
Oleic acid, methyl ester	-	-	-	4.34	-	-
Oleic acid, propyl ester	-	6.33	-	-	-	-
Oxalic acid, heptadecyl hexyl ester	-	-	-	1.71	-	-
Pentacosane	-	-	-	3.86	-	-
Phytol	-	-	2.2	-	-	3.44
Retinol, acetate	-	-	-	1.69	-	-
Solanesol	-	-	-	-	12.78	-
Squalene	3.74	-	-	4.65	-	-
Stigmasta-5,22-dien-3-ol, (3.beta.,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

Table 18: Bioactive compounds detected in the roots of non-AM and AM plants of *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses.

Bioactive compounds	Area percentage (%)					
	Non-AM	AM	Non-AM+Cd	AM+Cd	Non-AM+Zn	AM+Zn
Methyl palmitate	38.42	29.52	15.48	26.39	-	17.73
Gamma.-sitosterol	29.14	-	7.93	-	6.14	-
1,2-benzenedicarboxylic acid	9.66	14.74	7.26	11.98	21.81	25.98
1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	-	6.78	-	-	7.57	-
10-nonadecanone	-	-	-	-	-	3.98
Octadecenoic acid, methyl ester	6.87	12.42	3.63	8.39	8.94	10.13
1-heneicosanol	3.53	-	-	3.03	1.06	7.49
2-methyloctacosane	-	-	-	1.75	-	-
2-Pentadecanone, 6,10,14-trimethyl-	-	1.82	-	-	-	-
3beta-acetoysgmasta-4,6,22-triene	-	2.57	-	2.57	-	2.62
4,22-Stigmastadiene-3-on	-	-	2.26	-	-	-
8-octadecanone	-	-	-	-	-	4.92
9,12-Octadecadienoic acid, methyl ester	-	5.1	-	-	5.35	-
9-eicosene, (e)-	-	-	-	-	1.79	-
Cholesta-4,6-dien-3-ol, benzoate, (3.beta.)-	-	1.54	-	1.87	2.03	-
Cyclopentanone, 3-(2-oxopropyl)-	-	-	5.02	-	-	-
E-15-Heptadecenal	-	-	-	-	-	7.66
Eicosane	-	4.29	2.12	-	1.68	2.41

Eicosane, 2-methyl-	-	-	-	2.5	-	-
Ethyl iso-allocholate	-	-	4.51	-	-	-
Gamma-sitosterol	29.14	-	7.93	-	6.14	-
Heptadecane	-	3.78	-	-	-	-
Hexadecanoic acid, methyl ester	-	-	-	-	16.5	-
Hexahydrofarnesyl acetone	-	-	-	-	4.05	-
Linoleic acid, methyl ester	-	-	-	4.2	-	-
L-Norvaline, N-(2-methoxyethoxycarbonyl)-, hexadecyl ester	-	-	-	2.89	-	-
Methyl 18-methylnonadecanoate	-	-	-	2.14	-	-
Methyl lignocerate		2.75	5.26	2.95	2.31	-
Methyl stearate	12.34	13.08	8.04	15.46	6.16	5.81
Neophytadiene	-	1.54	-	-	-	-
N-hentriacontanol-1	-	-	1.87	-	-	-
Nonadecane	-	-	-	2.33	-	-
Octadecane	-	-	-	-	-	2.192
Oleic acid, propyl ester	-	-	9.79	-	-	-
Pentadecane	-	-	-	-	1.7	-
Phytol	-	-	-	5.94	2.09	5.23
Phytol, acetate	-	-	-	1.34	1.17	-
Retinol, acetate	-	-	-	-	2.18	-
Solanesol	-	-	-	-	2.57	-
Stigmast-5-en-3-ol, (3.beta.,24s)-	-	-	12.31	-	-	-
Stigmasta-5,22-dien-3-ol	-	-	14.46	-	-	-
Tetracosane, 3-ethyl-	-	-	-	2.54	-	-
Tributyl acetylcitrate	-	-	-	1.7	1.73	1.91

Table 19. Bioaccumulation of Cd and Zn in non-AM and AM *Z. mays* associated with mycorrhiza exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Values are expressed as mean ± SE of three independent experiments. Different alphabetical letters indicates significant difference between treatments (Duncan's test, $p \leq 0.05$). BDL-below detectable level

Treatments		Bioaccumulation of Cd and Zn (mg g^{-1} DW)											
		0 d			4 d			8 d			12 d		
		Root	Leaf	TF	Root	Leaf	TF	Root	Leaf	TF	Root	Leaf	TF
Non-AM	Cd	BDL	BDL	-	BDL	BDL	-	BDL	BDL	-	BDL	BDL	-
AM	Cd	BDL	BDL	-	BDL	BDL	-	BDL	BDL	-	BDL	BDL	-
Non-AM+Cd	Cd	BDL	BDL	-	0.6126 ± 0.031^{b}	0.4326 ± 0.022^{a}	0.71	5.0488 ± 0.252^{a}	0.6594 ± 0.031^{a}	0.13	3.3381 ± 0.167^{a}	0.515 ± 0.026^{b}	0.15
AM+Cd	Cd	BDL	BDL	-	0.7709 ± 0.039^{a}	0.2178 ± 0.011^{b}	0.28	3.7446 ± 0.187^{b}	0.6025 ± 0.033^{a}	0.16	2.6406 ± 0.132^{b}	0.6389 ± 0.032^{a}	0.24
Non-AM	Zn	0.0459 ± 0.002^{d}	0.0162 ± 0.001^{b}	0.35	0.0541 ± 0.003^{c}	0.0172 ± 0.001^{c}	0.32	0.0541 ± 0.003^{c}	0.0162 ± 0.001^{d}	0.30	0.0541 ± 0.003^{d}	0.0152 ± 0.001^{d}	0.28
AM	Zn	0.0614 ± 0.003^{b}	0.0274 ± 0.001^{a}	0.45	0.0703 ± 0.004^{c}	0.0261 ± 0.001^{c}	0.37	0.0703 ± 0.004^{c}	0.0281 ± 0.001^{c}	0.40	0.0703 ± 0.004^{c}	0.0251 ± 0.001^{c}	0.36
Non-AM+Zn	Zn	0.0541 ± 0.003^{c}	0.0152 ± 0.001^{b}	0.28	0.4417 ± 0.022^{a}	0.1558 ± 0.008^{a}	0.35	0.7409 ± 0.037^{a}	0.1222 ± 0.006^{a}	0.16	0.8093 ± 0.041^{a}	0.2984 ± 0.015^{a}	0.37
AM+Zn	Zn	0.0703 ± 0.004^{a}	0.0281 ± 0.001^{a}	0.40	0.2592 ± 0.013^{b}	0.1127 ± 0.006^{b}	0.43	0.251 ± 0.001^{b}	0.0888 ± 0.004^{b}	0.35	0.3588 ± 0.018^{b}	0.1942 ± 0.010^{b}	0.54

Table 20: Bioaccumulation of Cd and Zn (mg g^{-1} DW) in non AM and AM *O. sativa* associated with mycorrhiza exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Values are expressed as mean ± SE of three independent experiments. Different alphabetical letters indicates significant difference between treatments (Duncan's test, $p \leq 0.05$). BDL-below detectable level

Treatments		Bioaccumulation of Cd and Zn (mg g^{-1} DW)											
		0 d			4 d			8 d			12 d		
		Root	Leaf	TF	Root	Leaf	TF	Root	Leaf	TF	Root	Leaf	TF
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	-	0.3614 ± 0.02 [a]	0.6291 ± 0.001 [a]	1.74	4.293 ± 0.211 [a]	0. 748 ± 0.005 [a]	0.17	3.127 ± 0.163 [a]	0. 966 ± 0.003 [a]	0.31
AM+Cd		BDL	BDL		0.3772 ± 0.02 [a]	0.4677 ± 0.003 [b]	1.24	3.221 ± 0.160 [b]	0. 694 ± 0.048 [b]	0.22	2.255± 0.115 [b]	0.821± 0.005 [b]	0.36
Non-AM	Zn	0.0827 ± 0.008 [b]	0.0708 ± 0.002 [b]	0.86	0.0827 ± 0.005 [d]	0.0664 ± 0.001 [d]	0.80	0.0807 ± 0.011 [c]	0.0624 ± 0.001 [d]	0.77	0.0817 ± 0.002 [d]	0.0665 ± 0.015 [d]	0.81
AM		0.0997 ± 0.004 [a]	0.0885 ± 0.001 [a]	0.89	0.1028 ± 0.012 [c]	0.0885 ± 0.001 [c]	0.86	0.0993 ± 0.015 [c]	0.0885 ± 0.001 [c]	0.89	0.0922 ± 0.002 [c]	0.0885 ± 0.011 [c]	0.96
Non-AM+Zn		0.0827 ± 0.008 [b]	0.0664 ± 0.001 [c]	0.80	0.5937 ± 0.032 [a]	0.2148 ± 0.022 [a]	0.36	0.9184 ± 0.050 [b]	0.3659 ± 0.004 [a]	0.40	1.5591 ± 0.082 [b]	0.2748 ± 0.031 [a]	0.18
AM+Zn		0.0997 ± 0.002 [a]	0.0885 ± 0.001 [a]	0.88	0.3275 ± 0.025 [b]	0.2013 ± 0.021 [b]	0.61	1.248 ± 0.062 [a]	0.2849 ± 0.003 [b]	0.23	1.8604 ± 0.091 [a]	0.2309 ± 0.021 [b]	0.12

Table 21: SEM-EDX microanalysis of the leaves and roots of *Z. mays* plants exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Values are expressed as mean ± SE of three independent experiments. Different alphabetical letters indicates significant difference between treatments (Duncan's test, $p \leq 0.05$). BDL-below detectable level

Treatments		Treated metal (% weight)				
		leaves		roots		
		Spectrum 1	Spectrum 2	Spectrum 1	Spectrum 2	Spectrum 3
Non-AM	**Cd**	BDL	BDL	BDL	BDL	BDL
AM		BDL	BDL	BDL	BDL	BDL
Non-AM+Cd		3.62 ± 0.05^{b}	31.36 ± 3.6^{a}	20.12 ± 3.5^{b}	34.88 ± 8.1^{a}	22.80 ± 3.2^{b}
AM+Cd		2.2580 ± 3.2^{b}	35.5680 ± 3.2^{b}	12.53 ± 2.5^{c}	35.71 ± 6.1^{a}	28.20 ± 3.8^{b}
Non-AM	**Zn**	12.0280 ± 3.2^{b}	24.2580 ± 3.2^{b}	39.41 80 ± 3.2^{a}	30.80 ± 3.2^{b}	12.8680 ± 3.2^{c}
AM		16.2580 ± 3.2^{b}	23.2580 ± 3.2^{a}	11.5380 ± 3.2^{b}	14.8680 ± 3.2^{a}	10.1380 ± 3.2^{b}
Non- AM+Zn		19.32 ± 0.3^{a}	1.13 ± 0.06^{b}	37.68 ± 1.8^{a}	0.04 ± 0.002^{c}	22.22 ± 3.1^{b}
AM+Zn		10.2580 ± 3.2^{a}	5.6580 ± 3.2^{b}	13.1680 ± 3.2^{b}	42.8580 ± 3.2^{a}	16.2380 ± 3.2^{b}

Table 22: SEM-EDX microanalysis of the leaves and roots of *O. sativa* plants exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Values are expressed as mean ± SE of three independent experiments. Different alphabetical letters indicates significant difference between treatments (Duncan's test, $p \leq 0.05$). BDL-below detectable level

Treatments		Treated metal (% weight)				
		leaves		roots		
		Spectrum 1	Spectrum 2	Spectrum 1	Spectrum 2	Spectrum 3
Non-AM	Cd	BDL	BDL	BDL	BDL	BDL
AM		BDL	BDL	BDL	BDL	BDL
Non-AM+Cd		0.05 ± 0.003^{b}	0.08 ± 0.001^{a}	0.02 ± 0.001^{c}	3.76 ± 0.02^{b}	6.43 ± 0.001^{a}
AM+Cd		1.19 ± 0.04^{b}	4.47 ± 0.02^{a}	2.46 ± 0.03^{b}	10.46 ± 0.05^{a}	10.15 ± 0.02^{a}
Non-AM	Zn	8.26 ± 0.02^{b}	19.99 ± 0.03^{a}	0.02 ± 0.06^{c}	5.01 ± 0.02^{b}	7.44 ± 0.01^{a}
AM		0.05 ± 0.01^{b}	11.49 ± 0.05^{a}	22.51 ± 0.02^{a}	17.36 ± 0.02^{b}	10.95 ± 0.06^{c}
Non- AM+Zn		7.81 ± 0.02^{a}	6.14 ± 0.04^{a}	26.28 ± 2.6^{a}	3.10 ± 3.4^{c}	11.44 ± 2.4^{b}
AM+Zn		4.58 ± 0.08^{b}	16.55 ± 0.02^{a}	15.56 ± 0.06^{b}	20.79 ± 0.09^{a}	13.46 ± 0.02^{b}

Table 23: Cell wall thickening in the leaves and roots of non-AM and AM *Z. mays* and *O. sativa* plants exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Values are expressed as mean ± SE of three independent experiments. Different alphabetical letters indicates significant difference between treatments (Duncan's test, $p \leq 0.05$).

Sample	Treatments	Cell wall thickening (µm)		
		Xylem	Bundle cap	
***Z. mays* leaves**	**Non-AM**	3.021 ± 0.059 [a]	2.967 ± 0.09 [c]	
	AM	3.002 ± 0.059 [a]	2.857 ± 0.09 [c]	
	Non-AM+Cd	2.767 ± 0.148 [b]	3.567 ± 0.237 [ab]	
	AM+Cd	3.067 ± 0.148 [a]	3.067 ± 0.237[b]	
	Non-AM+Zn	1.967 ± 0.09 [c]	3.862 ± 0.189 [a]	
	AM+Zn	2.967 ± 0.09 [b]	3.1267± 0.189[b]	
***O. sativa* leaves**	**Non-AM**	1.837 ± 0.171 [b]	3.183 ± 0.348[b]	
	AM	1.857 ± 0.171 [b]	3.283 ± 0.348 [a]	
	Non-AM+Cd	2.267 ± 0.148 [b]	3.167 ± 0.448[b]	
	AM+Cd	1.967 ± 0.148 [b]	3.187 ± 0.448 [b]	
	Non-AM+Zn	2.867 ± 0.189 [a]	3.367 ± 0.323 [a]	
	AM+Zn	2.467 ± 0.189 [a]	3.167 ± 0.323 [b]	
		Endodermis	**Xylem**	**Epidermis**
***Z. mays* roots**	**Non-AM**	2.033 ± 0.09 [d]	2 ± 0.294 [e]	1.103 ± 0.062 [d]
	AM	2.043 ± 0.09 [d]	2.2 ± 0.294 [e]	1.203 ± 0.062 [b]
	Non-AM+Cd	3.433 ± 0.237 [a]	2.933 ± 0.301 [c]	1.167 ± 0.09 [c]
	AM+Cd	2.033 ± 0.237 [b]	2.333 ± 0.301 [d]	1.267 ± 0.09 [a]
	Non-AM+Zn	1.317 ± 0.162 [e]	4.1 ± 0.212 [a]	1.073 ± 0.038 [d]
	AM+Zn	2.317 ± 0.162 [c]	3.5 ± 0.212[b]	1.273 ± 0.038 [a]
***O. sativa* roots**	**Non-AM**	2.211 ± 0.155 [a]	2.133 ± 0.206[b]	0.897 ± 0.067 [c]
	AM	2.18 ± 0.142 [a]	2.143 ± 0.206[b]	0.917 ± 0.067[c]
	Non-AM+Cd	2.013 ± 0.009 [b]	3.4 ± 0.155 [a]	3.333 ± 0.897 [a]
	AM+Cd	2.1 13 ± 0.009[b]	3.2 ± 0.155 [a]	3.433 ± 0.897 [a]
	Non-AM+Zn	2.30 ± 0.256 [a]	3.333 ± 0.448 [a]	1.733 ± 0.179 [b]
	AM+Zn	2.34 ± 0.256 [a]	3.303 ± 0.448 [a]	1.833 ± 0.179 [b]

Table 24: Impact of Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses on root infection, vesicle and arbuscule development of *C. claroideum* associated with *Z. mays* roots. Values are expressed as mean ± SE of three independent experiments. Different alphabetical letters indicates significant difference between treatments (Duncan's test, $p \leq 0.05$).

Sample	Root infection (%)	Presence of arbuscules (%)	Presence of vesicles (%)	Diameter of vesicles (µm)	Presence of spores (%)
		4 d			
AM	98 ± 2^{a}	51 ± 2^{b}	85 ± 3^{b}	7.8 ± 0.02^{c}	41 ± 3^{b}
AM+Cd	99 ± 1^{a}	81 ± 8^{a}	90 ± 2^{a}	14.5 ± 0.06^{a}	50 ± 2^{a}
AM+Zn	98 ± 8^{a}	82 ± 4^{a}	88 ± 5^{a}	13.03 ± 0.05^{a}	53 ± 2^{a}
		8 d			
AM	100 ± 5^{a}	75 ± 2^{b}	87 ± 2^{b}	11.45 ± 0.06^{b}	46 ± 7^{b}
AM+Cd	98 ± 3^{a}	87 ± 3^{a}	98 ± 5^{a}	15.72 ± 0.01^{b}	82 ± 6^{a}
AM+Zn	100 ± 2^{a}	82 ± 2^{a}	99 ± 8^{a}	22.39 ± 0.04^{a}	75 ± 2^{a}
		12 d			
AM	100 ± 3^{a}	52 ± 2^{b}	90 ± 2^{b}	11.45 ± 2^{c}	59 ± 2^{b}
AM+Cd	99 ± 4^{a}	80 ± 5^{a}	99 ± 6^{a}	14.72 ± 1.8^{b}	80 ± 5^{a}
AM+Zn	100 ± 8^{a}	84 ± 8^{a}	98 ± 1^{a}	13.39 ± 1.5^{a}	76 ± 3^{a}

Table 25: Impact of Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses on root infection, vesicles, and arbuscule development of *C. claroideum* associated with *O. sativa* roots. Values are expressed as mean ± SE of three independent experiments. Different alphabetical letters indicates significant difference between treatments (Duncan's test, $p \leq 0.05$).

Sample	Root infection (%)	Presence of arbuscules (%)	Presence of vesicles (%)	Diameter of vesicles (µm)	Presence of spores (%)
			4 d		
AM	99 ± 2[a]	74 ± 9[b]	84 ± 5[a]	7.8 ± 0..01[b]	45 ± 5[b]
AM+Cd	99 ± 6[a]	80 ± 6[a]	85 ± 2[a]	14.5 ± 0.03[a]	50 ± 6[a]
AM+Zn	98 ± 5[a]	82 ± 1[a]	88 ± 8[a]	13.03 ± 0.02[a]	53 ± 2[a]
			8 d		
AM	99 ± 3[a]	52 ± 8[b]	87 ± 3[b]	11.45 ± 0.02[b]	46 ± 1[c]
AM+Cd	98 ± 1[a]	88 ± 5[a]	90 ± 8[a]	15.72 ± 0.05[b]	62 ± 4[a]
AM+Zn	100 ± 2[a]	80 ± 6[a]	95 ± 6[a]	28.39 ± 0.08[a]	51 ± 2[b]
			12 d		
AM	98 ± 4[a]	84 ± 3[b]	90 ± 2[b]	11.45 ± 0.05[b]	59 ± 4[b]
AM+Cd	99 ± 6[a]	92 ± 5[a]	99 ± 3[a]	16.72 ± 0.02[b]	72 ± 2[a]
AM+Zn	100 ± 8[a]	91 ± 6[a]	98 ± 8[a]	32.39 ± 0.03[a]	66 ± 4[a]

Table 26: Tolerance index and mycorrhizal dependency of non-AM and AM plants of *Z. mays* and *O. sativa* exposed to Cd (0.45 g kg^{-1}) and Zn (1.95 g kg^{-1}) stresses. Values are expressed as mean ± SE of three independent experiments. Different alphabetical letters indicates significant difference between treatments (Duncan's test, $p \leq 0.05$).

Treatments	*Z. mays*		*O. sativa*	
	Tolerance index (%)	Mycorrhizal dependency (%)	Tolerance index (%)	Mycorrhizal dependency (%)
Non-AM	-	-	-	-
AM	-	51.61 ± 0.895 [b]	-	17.54 ± 0.182 [c]
Non-AM+Cd	62.19 ± 0.443 [d]	-	82.32 ± 0.366 [d]	-
AM+Cd	77.79 ± 0.482 [b]	61.32 ± 1.092 [a]	90.19 ± 0.517 [c]	24.73 ± 0.811 [b]
Non-AM+Zn	67.98 ± 0.168 [c]	-	101.57 ± 0.79 [b]	-
AM+Zn	91.01 ± 0.909 [a]	63.86 ± 0.444 [a]	116.65 ± 1.89 [a]	28.20 ± 0.155 [a]

DISCUSSION

The contamination of agricultural lands with HMs is a growing concern of human population due to the biomagnification potential of these elements in the food crops (Wang et al., 2019). *Zea mays* and *O. sativa* are the two important staple foods for half of the world population and its production has to be increased to meet the growing demand. The cultivation of these crops in the HMs contaminated agricultural lands result in the uptake and translocation of these toxic metals to the shoot (Uraguchi et al., 2009; Gu et al., 2012; Rizwan et al., 2018). The HMs accumulation significantly hinders the metabolic processes in shoot resulting in the reduction of yield in the plants (Wang et al., 2019).

Cadmium is a non essential element widely available in the soil by anthropogenic smelting and mining activities. The exposure of plants to high concentrations of Cd affects growth and development of plants. High levels of Cd cause visible injuries lik chlorosis, growth inhibition, and finally lead to death of the plants (Wojcik and Tukiendorf, 2004). Cadmium induces generation of ROS and cause lipid peroxidation, indicated by higher accumulation of malondialdehyde (Singh and Shah, 2014). Cadmium also affects photosynthesis by down regulating PSII activity (Krupa et al., 1993). At the same time Zn, one of the essential elements turns toxic to plants only when its concentration exceeds the tolerance limit. When Zn is over accumulated in the plants, it induces detrimental effects by causing metabolic imbalances, affecting electron migration and decreasing membrane permeability (Rout et al., 2019). Zinc triggers the overproduction of ROS causing oxidative stress by the imbalance of antioxidation machinery (Cui and Zhao, 2011; He et al., 2016). Therefore, both these metals significantly reduce the yield and productivity of the crop plants.

Selection of low metal accumulating cultivars with the potential to grow in contaminated land, have been successfully utilized for extending the cultivation of *Z. mays* and *O. sativa*. The introduction of` microbial population with HM stress tolerance potential, to the roots of *Z. mays* and *O. sativa* will be an efficient strategy to increase Cd and Zn stabilization and metal tolerance potential of these plants. Arbuscular mycorrhiza is considered as a promising candidate for achieving the metal stabilization in rhizosphere and it reduces the translocation of metals to the above ground portions and thus to the food chain. At the same time, these organisms potentially enhance the metal tolerance of the crop plants. The present study validates the heavy metal stress tolerance of *Z. mays* and *O. sativa* through specific physiological, metabolic and anatomical dynamisms as a result of AM association. Moreover, this study also compares different tolerance mechanisms activated in mycorrhiza associated *Z. mays* and *O. sativa* to tolerate Cd and Zn toxicity.

5.1 Root colonization by arbuscular mycorrhizae

In the present study, the initiation of the root colonization by AM was observed on 4 d. The frequency of root colonization gradually increased and finally attained 100% in *Z. mays* and *O. sativa* by 45 d. The crop plants, *Z. mays* and *O. sativa* shows association with different species of *Glomus* and other mycorrhizal fungus (Boucher et al., 1999; Bhattacharjee and Sharma, 2011). Cereals were proved as most efficient host on the basis of their spore production efficiency and it supports our results (Simpson and Daft, 1990). Moreover, the host specificity and spore production efficiency of mycorrhizae was proved highly successful in barley associated with *G. mosseae* (Al-Raddad, 1995). According to the percentage of infection, *Z. mays* was found as the best host for AM association as compared to *O. sativa* and *Z. mays* was generally used as a major host in mycorrhizal propagation (Begum et al., 2019). The fungal compatibility of *Z. mays* hybrid Pioneer 3905 was

scrutinized with the association of four different AM fungi *viz. G. aggregatum, G. etunicatum, G. mosseae,* and *G. versiforme.* After 84 d of growth, these fungi showed 26-72% of association with the roots of *Z. mays* (Boucher et al., 1999). Of these fungi, *G. etunicatum* showed lowest rate of association as compared to others. Similar to this work, the differential ability of different *Glomus* species for colonization in *Allium porrum* was analysed and found that *G. intraradices* showed high rate of colonization as compared to *G. etunicatum* (Brundett et al., 1985). But, in the present study, both *C. claroideum* and *C. etunicatum* showed rapid hyphal ramification in *Z. mays*, may be due to the influence of host characters, quantity of inocula and growth conditions on the rate of colonization.

Arbuscular development exhibited high rate of fluctuations, indicating the formation and degeneration of arbuscules in a cyclic manner. Toth and Miller (1984) reported the formation, degeneration of arbuscules in a definite cycle, and they could depict the arbuscular cycle in *Z. mays* plant. According to their study *Z. mays* roots contained arbuscules at very early stages of development, *i.e.* 2 d after the infection. In the present study, the formation of arbuscules was delayed in *O. sativa*, but in *Z. mays* associated with *C. claroideum* the initiation of arbuscular cycle started on 4 d. This result was correlating with the initiation stages of arbuscular cycle in *Allium porrum* wherein it was observed that the arbuscular development initiated on 3-4 d of AM association (Brundett et al., 1985). According to the study of Alexander et al. (1988), the development of arbuscules started on 2.5 d, further, with in 12 h the arbuscules were entering into the early stages of degeneration.

When colonization process has reached a steady state, the arbuscule formation was observed to attain equilibrium with degeneration and it is known as phase of constancy by Sutton (1973). In the present study 70% frequency of arbuscules (*i.e.* 70 number of the 100 root bits having arbuscule

development) were observed during 4-20 d which gradually decreased to 10-25% by 24-28 d. These results indicate the existence of degeneration phase of arbuscules. Later a drastic increase occured in the formation of arbuscule and this type of arbuscular cycle was observed in *Z. mays* and *O. sativa* with the association of both *C. etunicatum* and *C. claroideum*. Arbuscules are structurally different and found in two form *i.e.* arum and paris types. Generally, arum type of arbuscules were observed in *Z. mays* and *O. sativa* and similar results were reported in barley, millet and ryegrass colonized by *G. coronatum* or *G. intraradices* (Smith and Smith, 1997).

Generally, vesicle is said to develop immediately after the development of arbuscules, but here vesicles were observed 24-32 d after inoculation, which increased to 84-100% on 44 d. These vesicles were selected as a good source of inoculum for the establishment of monoxenic cultures (Declerck et al., 1998). Similarly in another study, the intraradical vesicles of *G. fasciculatum* and *G. mosseae* showed high rate of proliferation in the roots of subterranean clover (Biermann and Linderman, 1983). More than this, vesicles are considered as the storehouse of fatty acids and lipids in the mycorrhizal body, which are the major source of food of these organisms (Jabaji-Hare, 1988). The development of vesicles and spores of *Funneliformis mosseae* was observed in the roots of *Z. mays* plants on 49 d of AM association (Müller et al., 2017). In the results of the present study, vesicles were developed on 24-32 d of plant growth and fast vesicular development was observed in *O. sativa* as compared to *Z. mays* and the formation of vesicles was increased to 74-100% on 45 d of association. Similar to vesicles, frequency of spores also showed a progressive increase in the roots of *Z. mays* plants. These spores were considered as the major component of the inoculum causing rapid colonization and development of spores significantly increase the infection rate of AM fungi in *Z. mays* plants (Müller et al., 2017). These spores show morphological similarities with vesicles and sometimes

distinguishing these two structures becomes difficult (Morton, 1985). The results of the study shows a similar pattern in the development of spores and vesicles indicating similarity in the pattern of development, which supports the hypothesis that vesicles are precursors of spores, put forwarded by Gerdemann and Trappe (1974).

5.2 Determination of optimal quantity of AM inoculum

The quantity of AM inoculum has a significant role in the rate of mycorrhizal colonization. In this study, a progressive increase was observed in the root colonization with an increase in the quantity of inoculum. The hyphal mass, vesicles, and spores constituted the inoculum for successful establishment of mycorrhizal association (Müller et al., 2017). In the present work, it was observed that, as the number of spores increases in the inoculum, the rate of mycorrhizal association was also increased. When the seedlings of *Andropogon gerardii* were treated with 0, 10, or 20 spores of *G. etunicatum* and 0, 10, or 20 spores of *G. intraradices*, the maximum mycorrhizal association was observed in the roots treated with 20 numbers of spores (Gustafson and Casper, 2006). This indicates that, the quantity of inoculum and the spores contained in it are important to establish maximum rate of AM association. The quantity of the inoculum and growth stage of the host plant were considered as the most important features determining the colonization potential of AM fungus (Simpson and Daft, 1990). In the present study, for AM inoculation 20 g of *C. claroideum* inoculum containing spores (approximately 320 spores per gram), hyphae, and AM infected host roots were introduced into the soil and it caused 95-100% of mycorrhizal colonization within 45 d of plant growth.

5.3 Selection of heavy metal tolerant mycorrhizal species

The major impact of heavy metal toxicity on mycorrhizae was the reduction in the spore germination and host colonization (Gildon and Tinker, 1983). Current study showed that the spore germination and colonization of plants were reduced in the mycorrhizae associated with both plants on exposure to high concentrations of HM. The diversity in the HM tolerance potential of different mycorrhizal species has to be analysed for the selection of best candidate for bioremediation process. The characterization of different *Glomus* species isolated from metal-contaminated sewage sludge proved that *G. claroideum* showed a high level of metal tolerance (del Val et al., 1999). The potential of *G. claroideum* in As and Zn stresses tolerance was analysed in *Eucalyptus globulus* and *Fragaria vesca* respectively (Turnau et al., 2001; Arriagada et al., *2009). Glomus claroideum* were colonizing 40% of the *F. vesca* roots collected from Zn contaminated sites, whereas *G. mosseae* and *G. intraradices* showed only 10-20% of colonization (Turnau et al., 2001). In the case of *E. globulus*, the As uptake to the shoot system was prevented with the association of *G. claroideum* as compared to *G. deserticola*.

The results of present study confirmed the high Cd and Zn tolerance level of *C. claroideum* over *C. etunicatum* based on the analysis of germination rate of spores, efficiency of root infection and total protein content in the rhizosphere. Similar results were observed in the roots of *Festuca rubra* and *Plantago lanceolata* cultivated in Zn contaminated land, where in *C. etunicatum* showed low frequency of colonization as compared to *C. claroideum* (Orłowska et al., 2005). The high metal tolerance level of *G. claroedonium* BEG133 was positively correlated to the cation exchange capacity of the extraradical hyphae of this fungus. Moreover, this fungus accumulated excess heavy metals in the mucilaginous outer hyphal wall zone, cell wall and inside the hyphal cytoplasm, which significantly reduces the

bioavailability of the metal to the plants (Gonzalez-Chavez et al., 2002). *So C.* claroideum was selected for the further part of this study.

5.4 Selection of stress imparting Cd and Zn concentrations

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 both *Z. mays* and *O. sativa* on 8 d of exposure. 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 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^{-1} (Mitra et al., 2018). Whereas, the concentration of Cd 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^{-1} of Zn caused growth retardation in 105 days old *Lycopersicon esculentum* (Vijayarengan and Mahalakshmi, 2013). These variation 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).

5.5 Impact of mycorrhization and heavy metal toxicity on the growth parameters

AM association induced increase in plant growth of *Z. mays* and *O. sativa* as compared to plants without 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). Similarly, the shoot fresh weight of *O. sativa* was also found to

increase due to mycorrhizal association (Ruiz-Sánchez et al., 2010; Xiao et al., 2010; Liu et al., 2013). Mycorrhizal associated enhancement in growth of plants could be due to several reasons. According to Tarraf et al. (2017), the growth enhancement observed in AM plants was due to efficient P absorption. The increase in biomass production of AM associated plants can be also attributed to the enhancement of photosynthesis rate (Frew, 2019). In the present study, root fresh and dry weight of *Z. mays* and *O. sativa* associated with *C. claroideum* significantly increased. The association of three AM fungi, *G. versiforme*, *G. mosseae*, and *G. diaphanum* significantly enhanced the root dry weight of *O. sativa* (Zhang et al., 2005) and this result confirmed the findings reported in this study.

Shoot fresh weight of both AM and non-AM plants was lower than that of control under Cd and Zn stresses. Cadmium and Zn induced reduction in the fresh weight of *Z. mays* was reported in different studies (Krantev et al., 2008; Dresler et al., 2015). There was a reduction in the fresh weight and dry weight of *O. sativa* on exposure to Cd and Zn toxicity (Song et al., 2014a). Under heavy metal stresses, fresh weight and dry weight of the plants reduces due to the reduction in the plant growth and cell division (Latef, 2018; Weisany et al., 2012). The reduction in the shoot fresh weight could also be attributed to the blockage in the xylem vessels, which hinder the transport of water and minerals towards the shoot (Subramanian et al. 2009). Moisture content in the leaf tissues of *O. sativa* and *Z. mays* was reduced due to the exposure towards Cd and Zn stresses. Similar results were observed by Mostofa et al. (2015) in *O. sativa* under Cd toxicity and this reduction in moisture content leads to the curling of leaf in *O. sativa* plants. Parallel results was observed in *Z. mays* due to Zn toxicity, where the moisture content showed a progressive reduction with an increase in the Zn toxicity (Janeeshma et al., 2021a). In the present study, mycorrhization mitigated the reduction in fresh weight and dry weight of shoots of plants subjected to Cd

and Zn stresses. Because in AM plants the water transport to shoots was not hindered as that of non-AM plants. Similar results were observed in *Helianthus annuus* exposed to Cd stress where *Funneliformis mosseae*, *Rhizophagus intraradices* and *C. etunicatum* were the AM fungal partners (Abd-allah et al., 2015).

The reduction in root fresh weight was attributed to the reduced cell division due to the exposure of toxic metals. In the case of dry weight, HMs induced reduction was prominent in *O. sativa* and *Z. mays.* The moisture content in the roots of *O. sativa* and *Z. mays* was increased under severe stress, indicating decaying or degeneration of cells. The increase of water content in the roots was directly related to the 'root dilution mechanism' wherein more water is taken up into the cells in order to dilute the high metal ion concentration in tissues and thus the plant tries to avoid the toxicity. This root dilution mechanism was predominant in non-AM plants than AM plants to withstand the elevated concentrations of Cd and Zn. The absorbed water might be stored in the vacuoles to reduce the concentration of accumulated metal ions (Rucińska-Sobkowiak et al., 2013). Under extreme stress, the complete water transport of the plant system become imbalanced due to metal induced cell wall thickening, reduced rate of transpiration, and blockage in xylem vessels (Rucińska-Sobkowiak, 2016; Janeeshma et al., 2020), and this was evidenced in non-AM plants. In the present study, it was clear that mycorrhization aid to alleviate the reduction in shoot moisture content and it prevents the excess of water storage in root cells. This may be due to the potential of mycorrhizae to maintain the transpiration rate similar as control even under suboptimal conditions, where this transpiration pull could aid for the transfer of water from root to shoot (Miransari, 2010).

As observed in many other cases, increased lateral root formation recorded in AM associated plants as compared to non-AM plants, was due to

enhancement in the rate of auxin biosynthesis in the former as compared to latter (Kaldorf and Ludwig-Müller, 2000). In contrast, heavy metal toxicity caused a significant reduction in the root area with a reduction in root hair surface in *Salix caprea* (Vaculík et al., 2012b). When *O. sativa* cultivars cv. TY-167 and cv. FYY-326 were treated with high concentrations of Zn, significant reduction in root area was observed (Song et al., 2011). In the present study, mycorrhizae mediated alleviation in root volume reduction was clearly observed in *Z. mays* and *O. sativa*. Similar response of reduced root volume was observed in a study conducted by Rohani et al. (2019), where the association of *Glomus mosseae* improved the root growth and volume of *Pistacia vera* under Cd toxicity. Modification of root architecture by mycorrhizal association was prominent in *O. sativa* plants (Chen et al., 2017). Root volume, root length, number of root hairs, and root surface area were the different traits increased in the host plant by the association of *G. mosseae* under different abiotic stresses (Chen et al., 2017).

5.6 Effects of AM on photosynthesis of *Z. mays* and *O. sativa* exposed to Cd and Zn stresses

Cadmium and Zn toxicity reduced the photosynthetic efficiency of both non-AM and AM plants by reducing chlorophyll and carotenoids content, electron transport efficiency and stomatal opening. Both heavy metals, Cd and Zn affected the integrity of photosynthetic apparatus and reduced the rate of photochemical reactions by inducing the degradation of chlorophyll molecules in *Z. mays* and *O. sativa* as reported in *Cucumis sativus* (Feng et al., 2010), *Trigonella foenum-graecum* (Bashri and Prasad, 2015), *Elsholtzia argyi* (Li et al., 2015) and *Triticum aestivum* (Ci et al., 2010). Cadmium toxicity prevents the synthesis of chlorophyll by the replacement of Mg^{2+} with Cd^{2+}. Moreover, Cd toxicity leads to a decrease in the biosynthesis of chlorophyll and it inhibits the activity of enzymes involved in Calvin cycle

(Baryla et al., 2001). A the same time, Zn toxicity caused reduction of chlorophyll content in non-AM and AM plants was reported in *Miscanthus* × *giganteus* plants (Andrejić et al., 2018). The reduction in total chlorophyll content in *Z. mays* and *O. sativa* under Cd and Zn stresses can be attributed to the reduction in the rate of chlorophyll biosynthesis, due to the metal induced inhibition of protochlorophyllide reductase and δ-aminolevulinic acid dehydrogenase (Kasim, 2007; Rana and Kashif, 2013).

As compared to *O. sativa*, a drastic reduction in total chlorophyll content and carotenoids was observed in the *Z. mays* leaves on exposure to Cd and Zn stresses causing photosynthetic inefficiency and was one of the prime reasons for decreased tolerance of *Z. mays* towards these metals. Results clearly showed that in AM plants, the reduction of chlorophyll and carotenoid content was lesser as compared to non-AM plants under HM stress. Similarly, AM aided in the maintenance of chlorophyll content in *Cajanus cajan* under Cd and Zn stresses (Garg and Singh, 2018). The negative impact of hexavalent chromium (Cr^{+6}) on *Brachiaria mutica* was reflected in the reduced chlorophyll and carotenoid content, but the association with *Rhizophagus irregularis* improved the chlorophyll and carotenoid content (Kullu et al., 2020). The lower concentration of Cd and Zn detected in the leaves of AM plants due to the mycorrhizae mediated stabilization of metals in roots, helps the AM plants to avert the excess presence of metal ions in leaves which aid to overcome the inhibition in the chlorophyll biosynthesis. These results could be related to the findings obtained from *Phragmites australis* under metal-stressed conditions, where metal induced inhibition in photosynthesis by degrading the photosynthetic pigments was mitigated with the association of *R. irregularis* (Wu et al., 2020).

The antenna complex was negatively affected with elevated metal concentrations and it was reflected as delay in energy migration to the

reaction centers. ABS/RC and ABS/CS_M are the two important parameters indicating the efficiency of antennae complexes for energy absorption and reduced values of both these parameters in *Z. mays* and *O. sativa* reveals Cd and Zn induced inefficiency of antennae complexes for effective energy absorption. Similar to this result, Cd and Zn induced reduction in absorption flux was seen in *Arabidopsis arenosa* and *A. halleri* (Szopiński et al., 2019). The very distinguishable reduction in the efficiency of antennae complexes in *Z. mays* acts as the primary limiting factor for successful photosynthesis and for this reason, it is considered as highly vulnerable to Cd and Zn stresses. Initial fluorescence (F_O) was reduced under Cd toxicity due to the degradation of reaction centers and the inefficiency in energy absorption (Vassilev and Manolov, 1999). The reduction of F_O in Cd treated *Z. mays* leaves could be considered as an indicator for the susceptibility of the plant towards Cd, whereas F_O value of *O. sativa* indicates better tolerance of this plant towards Cd stress. The reduction in F_M could also be correlated to the reduced chlorophyll content and PSII efficiency under the exposure of Cd and Zn stresses (Paunov et al., 2018).

Significantly, higher reduction of Fv/Fo in *Z. mays* over *O. sativa* indicates the higher functional impairment in the activity of the water-splitting complex on the donor side of PSII. Similarly, Fv/Fo was reduced in *Sedum alfredii* and *Sorghum-sudangrass* hybrid under Cd and Zn stresses respectively (Zhou and Qiu, 2005; Oh and Koh, 2016). Cadmium and Zn induced damage to the water-splitting complex of *Z. mays* was reflected in the reduced PSII activity, seen as reduced evolution of oxygen.

The efficiency of PSII reaction centres is one of the important factors determining the efficiency of electron flow in noncyclic photophosphorylation. The response of *O. sativa* and *Z. mays* plants towards Cd and Zn stresses was seen to cause a prominent reduction in RC/CS_M and it

was similar to that observed in durum wheat subjected to Cd stress (Paunov et al., 2018). F_V/F_M and PI_{ABS} were also reduced in metal treated *Z. mays* and *O. sativa* leaves, indicating that the efficiency and charge separation capacity of PSII was affected by the high concentration of metal ions. The F_V/F_M had a strong negative correlation with the accumulation of ROS and MDA, indicating the negative impact of oxidative stress induced by Cd and Zn on efficiency of photosynthetic machinery. Metal toxicity induced reduction in F_V/F_M and PI_{ABS} was observed in *Phaseolus vulgaris* (Wael et al., 2015), durum wheat (Paunov et al., 2018), *Helianthus annuus* (Azevedo et al., 2005) and *Brassica juncea* (Yusuf et al., 2010). Driving force (DF) indicates the importance of individual components in driving the processes in PSII. The drastic reduction in the DF of Cd treated *Z. mays* plants was mainly due to the reduction in the partial driving force for the conversion of excitation energy required for the electron transport beyond Q_A (Krüger et al., 2014).

The efficiency of electron migration depends on the rate of oxidation and reduction of plastoquinone. F_J depends on the availability of oxidized PQ-molecules bound to the Q_B-site of PSII and this could be also utilized to quantify the oxidoreduction state of Q_A and Q_B (Zhu et al., 2005; Tóth et al., 2007). In the present study, F_I and F_P were decreased in Cd and Zn treated *O. sativa* and *Z. mays* plants, which correspond to the state of $Q_A^-Q_B^{2-}$ and $Q_A^-Q_B^{2-}/PQH_2$ respectively. But F_J (corresponds to $Q_A^-Q_B/Q_A^-Q_B^-$ state) showed some species-specific response towards Cd, and it was reduced in *Z. mays* and increased in *O. sativa* as compared to the respective controls. This reduction in the F_J value denotes the Cd-induced reduction in plastoquinone pool and OEC and thus the primary photochemical events of *Z. mays* plants were extremely sensitive to the Cd toxicity as compared to the *O. sativa*. Closed reaction centers in the phenomenological energy pipeline model represents PSII reaction centers in which the Q_A was reduced. The density of closed

reaction centers was maximum in the *Z. mays* leaves treated with Cd, which indicates the incapability of Q_A to get oxidized.

The efficiency of the acceptor side of PSII molecule enhances with an enhancement in the turnover number (N), but Cd and Zn stresses cause a reduction of the same. This reduction in turnover number possibly indicates lesser number of excited electrons (Sayyad-Amin et al., 2014). Cadmium treated *O. sativa,* keep up the efficiency of electron migration by ensuring the turnover number same as that of the control, but the inefficiency of *Z. mays* to maintain the turn over number makes it more sensitive towards Cd and Zn stresses. Mycorrhization aided to improve the Cd tolerance of *Z. mays* by increasing the turn over number. The reduction in φEo value and ETo/RC also represents the Cd and Zn stresses induced impairment of e^- transfer from Q_A^- to plastoquinone.

The total e^- transfer efficiency of ETC was represented as PI_{TOTAL}, it takes into account the capacity of PSI as well as PSII (Yusuf et al., 2010). A reduction of this parameter indicates a loss in the efficiency of energy transfer as observed in the Cd affected *Z. mays* and *O. sativa* plants as well as in Zn affected *O. sativa* plants. Previous studies have showed similar results in *T. foenum-graecum* due to Cd stress (Bashri and Prasad, 2015).

The negative peaks observed in the IP phase of *Z. mays* and *O. sativa* directly correlates to the reduction in the density of PSI in both Cd and Zn treatments (Schansker et al., 2003). It also represents the high sensitivity of the PSI reaction center of *Z. mays* plants towards Cd, where the IP phase exhibited a dramatic reduction. tF_M has a strong correlation with the PSII/PSI ratio and the size of the PSI acceptor side pool (Kalaji et al., 2017). This parameter was increased in Cd and Zn treated *O. sativa* and Zn treated *Z. mays*, but it was decreased in Cd treated *Z. mays* plants. The reduction in F_M and the associated reduction in tF_M was reported in *Solanum tuberosum* due to

the reduction in PSII activity and density (Olechowicz et al., 2018), and similar results were observed in this study too. In the present study, Cd and Zn induced a reduction in the area above induction curves of *O. sativa* and *Z. mays* plants which indicates changes in the redox state of ETC or changes in the stoichiometry of PSII and PSI acceptor side (Schansker et al., 2011; Kalaji et al., 2016; Kalaji et al., 2017).

The reduction of ABS/CS_M, TRo/CS_M, and ETo/CS_M in *Z. mays* and *O. sativa* was related to the increased density of inactive reaction centers and the inefficiency of PSII and it strongly indicates the vulnerability of the plant towards elevated Cd and Zn content. Dissipation was decreased when it was analyzed in CS, but at the same time, it was increased per active RC in Cd treated non-AM plants of *Z. mays* indicating its high sensitivity. The increase in the DIo/RC was associated with increased dissipation of absorbed light as heat which specified the inefficiency in the utilization of energy (Kalaji et al., 2011; Faseela et al., 2019). But, the reduction in the DIo/CS_M was proportional to the reduction in the absorption due to the increase in the inactive RCs and this phenomenon was also prominently observed in *Z. mays* plants (Zushi et al., 2012).

Non-AM plants of *Z. mays* and *O. sativa* showed a prominent reduction in F_M, Fv/Fo, RC/CS_M, F_V/F_M, PI_{ABS}, reversion of reduced Q_A and DF as compared to mycorrhizal plants, indicating the potential of mycorrhizae to alleviate the impact of Cd and Zn stresses on reaction centers, photolysis of water, electron transfer, and PSII activities. Moreover, mycorrhization aided in mitigation of the Cd and Zn induced reduction of ABS/CS_M, TRo/CS_M, and ETo/CS_M in *Z. mays* and *O. sativa*, and thus the photosynthetic efficiency of AM plants was maintained under metal stress. Similar results were observed in *Triticum aestivum* cv. Sardari 39 plants associated with *G. mosseae* exposed to toxic levels of Cd (Shahabivand et al., 2012). In mycorrhizal

plants, the reduction in Fv/Fm was insignificant. Non-AM and AM plants of *Helianthus annuus* exposed to different concentrations of Cd showed reduction in $F_{M,}$ but the reduction was lower in plants associated with mycorrhizae (Shahabivand et al., 2017). Similarly, when *O. sativa* was exposed to Pb stress, Fv/Fo value was maintained in the endophytes associated plants (Li et al., 2012a). Similar results were observed in *Medicago truncatula* associated with *G. irregulare*, under Cd stress and these plants showed reduction in the photosynthetic yield parameters but the association of *G. irregulare* partially alleviated the negative effects (Aloui et al., 2011). All these results indicates that AM association aid to maintain the integrity of PSI and PSII and its proper functioning with improved electron transport efficiency (Shahabivand et al., 2012).

It is always a matter of confusion to select an appropriate parameter for distinct study of heavy metal stress effects in plants. But the results of this study have clearly showed that different JIP parameters; area, F_V/F_O, $F_V/F_{M,}$ and RC/CS_M showed significant changes and these parameters could be used to assess the intensity of stress as well as the tolerance potential of mycorrhizae associated *O. sativa* as well as *Z. may* under Cd and Zn stresses.

The reduction in PSII activity observed in *Z. mays* and *O. sativa* plants under metal exposure attributed to the inefficiency of OEC, inhibition in electron transport and structural damage to PSII due to the elevated metal ions. The observations supports the findings of the study conducted in barley plants exposed to Cd stress (42 mg kg^{-1}) (Vassilev et al., 2004). The higher level of Cd and Zn stresses tolerance of *O. sativa* was prominently observed in the results of PSII activity, where the Cd and Zn induced reduction in PSII activity was lesser than that of the *Z. mays*. Moreover, mycorrhization improved the PSII activity of *Z. mays* and *O. sativa* under Cd and Zn stresses. Parallel results were observed in Cd exposed *Medicago truncatula* associated

with *G. irregulare* (Aloui et al., 2011). The polarographic measurements corresponding to the O_2 consumption indicates a reduction in the activity of PSI under Cd and Zn toxicity in *Z. mays* and *O. sativa*. Similar results were observed in the experiments with barley plants exposed to different concentrations of Cd (Vassilev et al., 2004). The prominent reduction in PSI activity observed in *Z. mays* plants exposed to Cd and Zn stresses was again indicative of the low metal tolerance potential of *Z. mays* as compared to *O. sativa*. But mycorrhizal association significantly improved the PSI activity of *Z. mays* which was evidenced from the polarographic measurements and OJIP parameters. Maintenance of PSI activity would ensure the ATP synthesis via active photophosphorylation, which could be effectively used in energy requirement for stress tolerance.

Stomatal closure was the major limitation to photosynthesis under Cd and Zn stresses (Sagardoy et al., 2010). Cadmium induced reduction in leaf relative water contents and higher stomatal resistances was observed in *Phaseolus vulgaris* (Poschenrieder et al., 1989). In the present study, both Cd and Zn induced stomatal closure in non-AM and AM plants of *Z. mays* and *O. sativa*, but AM plants maintained partial stomatal opening and thus retained the ambient condition for maintaining photosynthetic efficiency. The hydro active closure of stomata is a direct effect of water loss and desiccation of plants (Stålfelt, 1955). So, the stomatal closure can be correlated with the tissue moisture content, which was drastically reduced in non-AM plants as compared to the AM plants. In the case of *Z. mays* a heavy loss of water was observed during Zn exposure, it resulted in the "wrong way" opening of stomata, that is the hydro passive stomatal opening (Arve et al., 2011). Here, *Z. mays* showed severe structural loss of stomata by the changes in the shape of guard cells under Cd stress, whereas *O. sativa* mainly responded toward the Cd toxicity by closing the stomatal pores due to heavy loss of water from leaf

tissues. But on exposure to Zn stress, a partial closure of stomata was observed indicating efficient water status and gas exchange in *O. sativa.*

Analysis of leaf micromorphological characters of *O. sativa* revealed the presence of circular shaped opening like special structures in Zn treated non-AM plants, which further reduced the photosynthetic efficiency. The structural modification in the leaf micromorphology was a common response of plants towards heavy metal stress (Rai and Mehrotra, 2008; Sruthi and Puthur, 2019). Ultramorphological changes such as reduction in the wax deposition and widening of stomatal opening was observed under Cr toxicity in *Phyllanthus amarus* (Rai and Mehrotra 2008). At the same time, Pb induced an increase in the number of stomata of soybean leaves (Weryszko-Chmiekwska and Chwill, 2005). The specific leaf modifications observed in the present study is being reported for the first time in *O. sativa* under Zn toxicity. Interestingly, it was not observed in *O. sativa* exposed to Cd toxicity. The Zn induced accumulation of volatile compounds could have led to the development of this structure, through which emission of these volatile organic compounds may be possible. Zinc potentially induced the volatile compound accumulation in *Martianthus leucocephalus* as compared to Cd (Jesus et al., 2016). Increase in the methanol, propanal, acetylene and acetaldehyde content in the leaves of *Tetradenia riparia* on exposure of Zn stress supports our hypothesis (Bibbiani et al., 2018). Otherwise, the total closure of stomata under Zn stress condition could have led to the development of an alternative structure in place of stomata or this structure may help to extrude the Zn malate, Zn citrate, and Zn phosphate like complexes from leaf epidermal cells (Saraswat and Rai, 2011). The absence of these structures in AM plants indicates the less toxicity due to lower Zn concentration in the leaf cytoplasm as compared to the non-AM plants.

5.7 Alleviation of Cd and Zn induced oxidative stress by mycorrhization

Heavy metal toxicity induces oxidative stress in plant cells by imbalancing different homeostatic events, which leads to accumulation of ROS (AbdElgawad et al., 2020). The generation of oxygen radicals in photosynthesis is a normal part of plant metabolism, but the overproduction of ROS because of Cd and Zn stresses may cause oxidation of D1 protein and entire PSII protein damage (Marshall et al., 2002). The higher $O_2^{\bullet-}$ in *O. sativa* indicates the inefficiency in the transfer of absorbed energy to PSII. The over-accumulation of H_2O_2 in *Z. mays* was due to the inadequate activity of peroxidase and this accumulated H_2O_2, which further reduced the rate of photosynthesis by inhibiting the electron transport (Samuilov et al., 2001). H_2O_2 induced inhibition in the electron transfer was studied in the cells of *Anabaena variabilis* and found that H_2O_2 potentially reduces the photosynthetic O_2 evolution and electron transport (Samuilov et al., 2001).

The outbreak of ROS was predominantly observed in *Z. mays* as compared to *O. sativa* and it points to the low Cd and Zn tolerance potential of *Z. mays*. Moreover, H_2O_2 overproduced in *Z. mays* plants caused a high rate of reduction in photosynthesis and it may be due to the formation of $OH^{\bullet}$. H_2O_2 reacts with $O_2^{\bullet-}$ to form more reactive $OH^{\bullet}$ in the presence of trace amount of Fe or Cu and this $OH^{\bullet}$ initiates self-propagating reactions leading to degradation of lipids and proteins, which finally leads to the structural deformation of chloroplast (Subba et al., 2014). Mycorrhization significantly reduced the accumulation of ROS in *Z. mays* and *O. sativa*. Mycorrhizae induced reduction in ROS content was due to the increase in the chelation of toxic metal ions, which reduce its bioavailability to plants, simultaneously this symbiotic association potentially elicit the production of different antioxidants in roots, which also helps to maintain redox homeostasis in the cytoplasm (Chen et al., 2004; Casarrubia et al., 2020).

Apart from this, ROS also elicit the peroxidation of lipids (Pospíšil et al., 2016) and from the present study, it was evident from the elevated MDA (product of lipid peroxidation) content in *Z. mays* and *O. sativa*. The increase of MDA content in *Z. mays* and *O. sativa* leaves signifies oxidative stress, resulting in increased leakage of cell contents during Cd and Zn stresses. Cadmium and Zn induced increase in the electrolytes leakage was prominently observed in the non-AM plants of *Z. mays* and *O. sativa.* Parallel results were observed in cotton leaves exposed to Cd stress, wherein electrolytes leakage was increased with the accumulation of ROS (Farooq et al., 2016). Similar to these results, Zn induced accumulation of ROS and MDA was reported in C*itrus reticulate* (Subba et al., 2014). Malondiadehyde is an indicator of lipid peroxidation caused due to the accumulation of ROS, as a result of HM toxicity (Lambardi and Sebastiani, 2005). When lipid peroxidation occurs via free radicals, numerous reactions are initiated which causes damage to free and membrane bound fatty acids and finally causes degradation of biological membranes (Giannakoula et al., 2021). In this study, an increase in MDA content was observed in AM and non-AM plants subjected to Cd and Zn treatment, but non-AM plants exhibited higher accumulation of MDA than AM plants, which indicates increased oxidative stress in the former. The enhancement in the level of $O_2^{\bullet-}$ and H_2O_2 content showed direct correlation with the enhancement of MDA content. Mycorrhization reduced the accumulation of $O_2^{\bullet-}$ and H_2O_2 content in leaves of *O. sativa* and *Z. mays*, accordingly MDA accumulation was also reduced in AM plants. The accumulation of MDA has a significant negative correlation with total chlorophyll content and PSII activity, which confirm the impact of the oxidative stress on photosynthesis.

MDA content was decreased in the roots under HM exposure and this could be correlated to the reduced live root cells, which was evidenced in the Evans blue staining of the root tissue. This azo dye has the potential to

penetrate into dead or non-viable cells. Similar results of reduced viable cells were observed in the *O. sativa* roots tissues exposed to Cu stress (Chen et al., 2004). The enhanced ROS content in the root tissues indicates the enhancement of the oxidative stress imparted by Cd and Zn toxicity and mycorrhization helps to reduce ROS generation and thus protect the root cells from oxidative stress. Mycorrhizal mediated oxidative stress alleviation was reported in *Trigonella* leaves exposed to Cd stress. MDA content was significantly increased with increasing Cd concentrations in the leaves of both AM and non-AM plants of *Trigonella*, but the level of MDA was low in AM plants as compared to non-AM plants (Abdelhameed and Metwally, 2019). Mycorrhization can be considered as one of the best strategies to avoid oxidative stress under Cd and Zn stresses.

5.8 Metabolic dynamism in tolerance of metal toxicity

5.8.1 Impact of mycorrhization on Cd and Zn induced alterations of primary metabolites

The drastic increase of sugar content observed in the leaves of *Z. mays* and *O. sativa* under Cd and Zn stresses indicates the upregulation of sugar metabolism to meet the higher carbon/energy requirement to cope up with the stress conditions (Rosa et al., 2009; Mishra et al., 2014). Cd induced enhancement of soluble sugar content in *Cajanus cajan* was reported by Garg and Chandel (2012) and they also observed higher sugar content in AM plants as compared to non-AM plants. In the present study, soluble sugar content was higher in the leaves of non-AM plants in the early stages of Cd and Zn stresses exposure. The soluble sugars accumulation was triggered in response to ROS outbreak and it stabilizes cellular membranes (Keunen et al., 2013). Therefore, the elevated ROS accumulation could be a major reason for the increased biosynthesis of sugar content in non-AM plants. The accumulation of sugars, even when there was a reduction in photosynthesis, under Cd and

Zn stresses could be largely contributed by the excessive degradation of starch (Dong and Beckles, 2019). But such a trend does not remain the same in *Z. mays* and *O. sativa*, with the increase in treatment period and concentration of the metal, 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 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 that synthesis process of these sugars are seriously hampered (Zoghlami et al., 2011). This phenomenon becomes more prominent in non-AM plants, where in 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 have the potential to 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.

At extreme stress, sugar content was reduced in roots of non-AM and AM plants of *O. sativa* and *Z. mays* on exposure to heavy metals, which was related to the reduction in sugar biosynthesis and allocation. Moreover, sugar formed by the starch degradation would be utilized in the shoot itself and the transportation of the same towards roots were critically reduced. On comparison between non-AM and AM plants, mycorrhizal associated roots had more sugar content on being subjected Cd and Zn stresses. Similar results were observed in the roots of mycorrhiza associated *Capsicum annuum* L. cv. Zhongjiao 105 exposed to Cd stress (Latef, 2018). It indicates the potential of mycorrhization to maintain the metabolic status of roots and shoots under Cd and Zn stresses in *Z. mays* and *O. sativa.*

Soluble protein content increased in the leaves of non-AM and AM plants subjected to metal stress, indicating the upregulation in the biosynthesis of enzymatic antioxidants and the stress induced proteins. 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). Mycorrhizae induced enhancement in the activity of different antioxidant enzymes involved in the ROS scavenging was observed in *Canavalia ensiformis* under elevated Cu stress (Andrade et al., 2010). However, in both non-AM and AM plants, there was a reduction in the protein content 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. Cadmium (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 protein synthesis process as similar as to the senescence stage of the plants (Zoghlami et al., 2011). When *Z. mays* plants with mycorrhizal association was exposed to Zn stress, the decrease in protein content was reduced and similar type of results were obtained in *S. lycopersicum* (Zoghlami et al., 2011).

At initial stages of HM stress, the response of *Z. mays* and *O. sativa* roots were similar, where the AM associated roots showed more protein content as compared to the non-AM plants. Similar results were observed in the roots of mycorrhizae associated *C. annuum* exposed to Cd stress (Latef, 2018). It indicates the potential of mycorrhization to maintain the protein biosynthesis in roots under Cd and Zn stresses in *Z. mays* and *O. sativa* that aid to tolerate the toxic effect of metal ions.

Accumulation of amino acids under heavy metal stress is a common strategy observed in different plants (Sharma and Dietz, 2006; Sruthi and Puthur, 2019). Plants exposed to HM toxicity contain high amount of N-metabolites which includes amino acids, peptides and amines and these

molecules has three major functions namely metal binding, antioxidant defense and signaling (Sharma and Dietz, 2006). 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. The major amino acids produced in plants during HM stress are proline and histidine. The accumulation of histidine was increased in Ni-hyperaccumulator *Allium* sp. during HM toxicity (Yusuf et al., 2011). When *Canavalia ensiformis* was exposed to copper stress, there was an increase in the amino acid content of non-AM plants than AM plants, at the early stages of treatment (Andrade et al., 2010), and was in line with the observations of this study. Increased proline and total amino acids content were observed in jack bean plants on exposure to elevated concentrations of Zn in the non-AM plants as compared to AM plants (Andrade et al., 2009). The increase in the amino acids accumulation indicates oxidative stress in plants on exposure of HM stress by the over accumulation of ROS (Andrade et al., 2010). But at later stages, the amino acids content was drastically reduced in the shoots of non-AM plants indicating Cd and Zn induced impairment in the amino acids biosynthesis. At the same time, mycorrhization aided in 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*, where the amino acid biosynthesis was maintained on exposure of Cd stress (Garg and Chandel, 2012).

Stability in the metabolic status of mycorrhizal roots was observed in the present study by the lower reduction in amino acids content of mycorrhizae associated *Z. mays* and *O. sativa* as compared to the drastic reduction observed in the non-AM plants. The elevated amino acids especially histidine, significantly contributes to the metal tolerance by the formation of histidine-metal complex. It was earlier reported that *Thlaspi*

goesingense exhibited increased root histidine concentrations under Ni toxicity (Sharma and Dietz, 2006). Another amino acid asparagine, also got accumulated, which has the potential to complex with the metal and thus reduce Zn toxicity in plants (Sharma and Dietz, 2006). So the enhanced root amino acids content by mycorrhization under Cd and Zn stresses could aid to improve the metal tolerance by immobilizing more metal ions in the roots.

Accumulation of proline in response to heavy metal stress in leaves is widely observed in different plants. Proline accumulation is considered as an indicator of HM toxicity (Girija et al., 2002). One of the major reasons for the accumulation of proline was due to the disturbance in the water balance caused by the accumulated Cd^{2+} in the vasculature tissues hindering the smooth conduction of water. Proline accumulation in plant tissue occurs as a result of decrease in proline degradation, and increase in proline biosynthesis and also by hydrolysis of protein (Charest and TonPhan, 1990). 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). In the present study, proline content of *Z. mays* and *O. sativa* leaves increased with corresponding increase in the accumulation of Cd and Zn in the tissues.

Non-AM plants accumulated more proline in the initial stages of metal exposure as compared to AM plants. At initial stages of metal stress, non-AM plants absorbed high level of Cd and Zn ions and translocated to the shoot, and to encounter the metal toxicity, more proline was accumulated in the leaves. When *Cassia italica* was exposed to Cd stress, non-AM plants accumulated more proline as compared to AM plants (Hashem et al., 2016b) and this was supportive to the results of this study. At later stages of metal exposure, proline accumulation was at higher level in AM plants and this

shows that proline biosynthesis process was less interrupted in the case of AM plants but in the case of non-AM plants, synthesis process of proline gets disturbed.

The proline content was dramatically reduced in the roots of non-AM plants exposed to Cd and Zn stresses. Similarly, when *O. sativa* was exposed to Cu stress, reduction in proline content of roots was observed and external application of proline helps to protect roots from the damage by Cu toxicity (Chen et al., 2004). But, in the present study mycorrhization lowered the reduction in the proline content under Cd as well as Zn toxicity which enhanced the metal tolerance potential of *Z. mays* and *O. sativa* plants. Association with AM fungi has been reported to induce proline synthesis in crop plants such as *Cajanus cajan, Helianthus annuus* and *Solanum lycopersicum* (Abeer et al., 2015*;* Hashem et al., 2016a; Garg and Singh, 2018). Mycorrhizae induced proline accumulation could be attributed to the inhibition of proline dehydrogenase and increase in the activity of proline biosynthetic enzymes (delta 1-pyrroline-5-carboxylate synthetase and glutamate dehydrogenase) (Garg and Baher, 2013).

The reduction of sugars, protein, amino acids and proline in the shoots of AM plants as compared to non-AM plants does not indicate reduced tolerance of AM plant towards HM stress. It has to be understood that AM plants are not encountering the same stress situation as that of non-AM plants, which is very clear from the lower MDA content recorded in the former as compared to later. When the stress impacts was less in AM plants, the demand for the over production of various metabolites to counter the stress were also less as reflected in this study. In the case of root, the reduction of metabolites accumulation has strong relation with the enhancement in the tissue damage by the oxidative stress elicited by Cd and Zn ions. Thus, a higher reduction in root metabolite contents was observed in non-AM plants

as compared to AM plants. The optimal metabolic content in AM plants indicates low intensity of oxidative stress in the root due to effective stabilization of metal ions.

5.8.2 Impact of mycorrhization on Cd and Zn induced alterations of 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, phenolic 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 was observed in the leaves of *Z. mays* and *O. sativa* on 4 d of metal exposure, which points towards the immediate role of this secondary metabolite in stress alleviation. Cd and Zn induced elevation of leaf phenolics compounds was reported in different plants (Chen et al., 2019). Phenolics can directly scavenge the ROS developed during the Cd and Zn stresses and also can act as a strong metal chelator which aid in the metal tolerance of plants (Kısa et al., 2016). In early stage of metal exposure, total phenolics content was increased in non-AM plants coinciding with the increase in the ROS production whereas AM plants showed comparatively less phenolics accumulation. Similar responses of non-AM and AM plants were observed in *Cassia italica* exposed to elevated concentration of Cd (Hashem et al., 2016b). But, on 12 d of exposure the phenolics content was higher in AM plants exposed to Cd and Zn indicating higher metal tolerance level of these plants. This is a clear evidence that the phenolics biosynthesis pathway proceeded uninterrupted in AM plants but not in non-AM plants under metal stress.

In roots generally the phenolics content decreases with exposure to metal stress as observed in the case of *Linum usitatissimum* roots exposed to 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 interruption in the biosynthesis processes of phenolics by the metals stresses. At the same time, the results showed the potential of mycorrhization to mitigate the negative impact induced by HMs on the biosynthesis of phenolics in the roots.

Flavonoids are phenolic compounds with dihydroxy B-ring in its structure and having the potential to 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). This increase in the flavonoids content under Cd and Zn stresses is a strategy to detoxify ROS molecules in the leaves of *Z. mays* and *O. sativa*. At earlier stages of the stress, mycorrhization insignificantly contribute 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., 2021a). 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 shoot with less interruption (Janeeshma et al., 2021a). Roots also showed an increase in the anthocyanin accumulation which aid to chelate the metal ions and to transform metal ions to inactive forms. The ROS accumulated

during heavy metal stress has a crucial role in the signaling of anthocyanins synthesis. A study conducted in ten *Arabidopsis* mutants showed that the ROS molecules trigger the up-regulation of anthocyanins biosynthesis and the corresponding regulatory genes, resulting in the increased production of anthocyanins (Xu et al., 2017). In the present study, as the concentration of Zn was increased in the leaves, the MDA and anthocyanins content was also increased. Therefore, the rate of accumulation of anthocyanins can be directly related to the intensity of the oxidative stress caused due to the increase in Zn concentration (Landi et al., 2013).

According to Park et al. (2012), the leaf colour modification arising out of the accumulation of anthocyanins was the result of the translocation of metal ions to the leaves. In the present study, synthesis of anthocyanins was increased with an increase in the bioaccumulation of Cd and Zn. Mycorrhization aid the plants to prevent the translocation of Cd and Zn to the shoot system by immobilizing these ions in roots and thus the intensity of anthocyanin accumulation was low in AM plants as compared to non-AM plants. Moreover, in *O. sativa*, prominent increase of anthocyanin content occured on 12 d of exposure to Zn stress, but the increase of anthocyanin was very less in *Z. mays* plants. The higher anthocyanin content in *O. sativa* on exposure to extreme metal stress indicates high tolerance level of this plant as compared to *Z. mays.*

Alkaloids are cyclic and nitrogen-containing compounds in plants, which gets 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). Similarly, both non-AM and AM plants showed increase in the alkaloids content on exposure to 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 *Z.*

mays plants but not in *O. sativa*. High concentration of Ni induced inhibition in alkaloid production was reported in *Catharanthus roseus* (Idrees et al., 2013). However, mycorrhization have the potential to further elicit the alkaloid production in plants (Copetta et al., 2006; Toussaint, 2007). But, in the present study mycorrhizal association did not significantly contribute towards the alkaloid production in shoots of both non-AM and AM plants on 12 d of imparting Cd and Zn stresses. In the case of roots, mycorrhization maintained the alkaloid level but non-AM plants failed to maintain the alkaloids biosynthesis and thus reduced the alkaloid content resulting in a lowered capacity of alkaloid induced antioxidant activity and related metal tolerance of these plants.

In the present study, both qualitative and quantitative differences were observed in phytochemical composition in leaves of both non-AM and AM *Z. mays* and *O. sativa*. Mycorrhization potentially induce 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 beta-linalool and gamma-sitosterol in the leaves of *Z. mays* subjected to Cd stress. The accumulation of sterols are very important to plants, and the interaction of sterols with phospholipids helps the plant cells to maintain plasma membrane fluidity and permeability during stress conditions (Aboobucker and Suza, 2019). The accumulation of 3beta-acetoxystigmasta-4, 6, 22-triene, which have a potential to 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 indicate that, there was no ionic imbalance in AM plants due to the lowered metal uptake.

Neophytadiene is a sesquiterpene with three consecutive isoprene units. Reduction in the concentration of neophytadiene and increase in the concentration of dihydroergosterol in plants under Cd treatment indicates that the degradation of neophytadiene aid in the biosynthesis of dihydroergosterol. Dihydroergosterol maintain the plasma membrane fluidity and permeability by interacting with the phospholipids and helps the plants to overcome the stress (Bartram et al., 2006; Aboobucker and Suza, 2019).

Phytol is the side chain of chlorophyll and gets released during the hydrolysis of this pigment. Two forms of phytol with two different retention time (RT) were detected in the leaves extract of AM and non-AM plants of *O. sativa* and *Z. mays*, (E)-phytol (RT-27.387) and phytol (RT-31.849). On exposure to Zn stress, phytol content was reduced in both *O. sativa* and *Z. mays.* Abiotic stress induced conversion of phytol into fatty acid phytol esters was reported by Lippold et al. (2012) and this could be the reason for the reduction in the content of phytol. The higher RT value of the second form of phytol represents stress induced incorporation of lengthy hydrocarbon chains in the fatty acids of thylakoid membrane (Lippold et al., 2012). In the present study, Cd and Zn stresses altered the ratio between phytol and (E)-phytol in both mycorrhizal and non mycorrhizal plants. Only the second form of phytol was detected in Cd treated non-AM plants of *O. sativa*. This indicates that the accumulation of fatty acid phytol esters occured with Cd stress. Whereas in Cd treated AM plants, both forms were detected. This results indicates that in *O. sativa,* mycorrhization influence the ratio between these two forms of phytols. In the case of *Z. mays*, the ratio of phytol and (E)-phytol was changed on the basis of metal ions and mycorrhization did not significantly influence this ratio. In Zn treated non-AM plants of *O. sativa*, the concentration of phytols were reduced which indicates higher vulnerability of these plants towards Zn toxicity over AM plants. Even under the high concentrations of Zn, non-AM and AM plants of *Z. mays* did not exhibit any drastic changes in

the phytochemistry, which indicates the less influence of bioactive compounds in the tolerance of *Z. mays* towards Zn stress.

In *O. sativa*, Zn induced accumulation of 3beta-acetoxystigmasta-4,6,22-triene, which have the potential to activate the plasma membrane H^+-ATPase, aiding the transportation of protons out of the cells (Aboobucker and Suza, 2019). Cyclopropaneoctanoic acid is a representative of cyclic fatty acid, determining the physicochemical characters of membrane and was characteristic of Zn treated non-AM plants of *O. sativa*. This compound was reported as a stress alleviator and could help to tolerate the oxidative stress developed in non-AM plants. The metal induced elicitation of various biocompounds described above was prominent in the shoots of non-AM plants and it could be due to the higher concentration of Cd and Zn ions translocated to shoot system of non-AM plants. These excess metal ions impart more severe oxidative stress, which demand the overaccumulation of different antioxidant molecules.

As root is directly interacting with the mycorrhizae and heavy metals, it showed drastic changes in the quality of secondary metabolites. Stigmasterol, was considered as a strong signal for cellular defence (Aboobucker and Suza, 2019) and in this study, it was detected in non-AM plants of *O. sativa* under Cd stress. Drought induced increase in the stigmasterol content was reported in *O. sativa* and it helps to increase the stress tolerance (Kumar et al., 2015). The presence of tributyl acetylcitrate in mycorrhizal plants of *O. sativa* under Cd stress indicates the overproduction and exudation of organic acids, and the role of this compound in the chelation of metal ions (Ma et al., 1997; Javed et al., 2017).

Under Cd stress non-AM plants produced elixene, hexahydrofarnesyl acetone and methyl palmitate, and all these compounds have antioxidant properties, aiding to boost the defence system of the plant (Zhang et al.,

2016b; Novriyanti et al., 2021). Under Zn stress, both non-AM and AM plants of *Z. mays* accumulated tributyl acetylcitrate, 1,2-benzenedicarboxylic acid and 3beta-acetoxystigmasta-4,6,22-triene, these compounds are with antioxidant properties and helps to tolerate the elevated level of Zn ions in plant. The linoleic acid was increased in mycorrhizal plants exposed to Cd stress, which is the precursor of jasmonic acid, a potent hormone inducing heavy metal tolerance in plants (Kontos and Spyropoulos, 1996). In the present study, prominent increase of retinol and hexahydrofarnesyl acetone was seen in the roots of non mycorrhizal plants under Zn stress. Hexahydrofarnesyl acetone, an aliphatic ketone has a potential role in the antioxidation machinery (Singh et al., 2009). Retinol was significantly increased in *Phaseolus vulgaris* under stress (Zengin and Munzuroglu, 2005). Therefore, these two bioactive compounds could aid to tolerate the oxidative stress induced by Zn.

5.9 Elicitation of antioxidant defence against Cd and Zn stresses

The antioxidant defence system of plants was elicited due to the overaccumulation of ROS under Cd and Zn stresses. Increase in the ascorbate is a strong indicator of Cd and Zn stresses intensity as its biosynthesis is elicited based on the quantity of ROS accumulated in the cell (Bielen et al., 2013; Roy et al., 2017). A drastic increase in ascorbate content was observed in *Z. mays* which indicates a higher imbalance in ROS production and scavenging as compared to *O. sativa*. The ascorbate-glutathione cycle plays a significant role in the ROS balancing and ascorbate directly and indirectly plays a major role in reducing the level of ROS molecules (Bielen et al., 2013). Another important antioxidant glutathione, also aid to scavenge the ROS molecules and the increase in the ascorbate and glutathione content is a common response of plants towards HM toxicity (Alsahli et al., 2020). In the present study, these two antioxidants showed a significant increase in the

leaves and roots of *Z. mays* and *O. sativa.* Mycorrhization improved the biosynthesis of ascorbate and glutathion under Cd and Zn stresses, it increases the substrate pool of antioxidant enzymes. In addition, the increase in these antioxidants was reflected in the antioxidants enzymes activity of AM plants on 12 d of stress.

Zea mays on exposure to Cd stress exhibited a prominent increase in the activity of APX, but this increase seems to be insufficient in *Z. mays* to overcome the oxidative stress induced by Cd as reflected from the accumulation of H_2O_2 and ascorbate (Sofo et al., 2015). On extreme metal stress, the APX activity of *Z. mays* exposed to Cd and Zn stresses was reduced as compared to the control. However, this was not the condition in *O. sativa*, which showed enhanced APX activity that indicates the higher tolerance of *O. sativa* towards Cd and Zn stresses. Metal induced increase in CAT activity was reported in barley (Song et al., 2014b), *Arabidopsis thaliana* (Corpas and Barroso, 2017), sunflower (Azpilicueta et al., 2008) and this enzyme reduces the impact of H_2O_2 in plants. Increase in the CAT activity of *Vigna radiata* by the association of mycorrhizae under arsenic stress was reported, and reduction in the quantity of H_2O_2 content was observed (Alam et al., 2019). It was noticed that non-AM plants failed to balance the ROS scavenging and antioxidant activity, whereas AM plants maintained the ascorbate and glutathione pool and the activity of different antioxidant enzymes even under extreme Cd and Zn stresses.

Zinc induced increase in the activity of SOD, CAT and APX was observed in *Canavalia ensiformis* (Andrade et al., 2009). The association with *G. etunicatum* aid to control the ROS accumulation by reducing the metal uptake and thus the activity of these enzymes was low in AM plants as compared to non-AM plants. But, in the later stages, the enzymatic activity was reduced in non-AM plants of *Z. mays* and *O. sativa,* where as

mycorrhization increased the activity of antioxidant enzymes. The reduction of the enzyme activity in non-AM plants could be largely due to the degradation of proteins with the intensification of stress. Mycorrhizae induces upregulation in the activity of SOD, CAT and APX as observed in *Lonicera japonica* associated with *Glomus versiforme* that potentially alleviate the oxidative stress elicited by Cd ions (Jiang et al., 2016). GPOX also showed prominent increase during metal exposure, and this enzyme oxidize aromatic electron donors such as guaiacol and pyragallol at the expense of H_2O_2 (Erofeeva, 2015). Similar to other enzymes, GPOX was also increased in *Z. mays* and *O. sativa* with an increase in ROS accumulation. It was observed that GPOX was one of the stable enzyme and the activity of the same increased even in extreme metal stress but at the same time the activity of other three enzymes were impaired in the leaves of *Z. mays* and *O. sativa* by the action of toxic metal ions. The findings indicate the improvement in the antioxidant defence system of *Z. mays* and *O. sativa* with the aid of mycorrhization, which potentially help the host plants to tolerate metal toxicity. The vulnerability of *Z. mays* towards Cd and Zn stresses could be due to the failure in maintaining antioxidant activities as compared to *O. sativa.*

5.10 Implications of metabolic changes in the osmolality of plants

Osmotic adjustment is considered as an important mechanism of plants to survive the HM toxicity. The turgour maintenance by adjusting the solute concentration of cell sap significantly contributes towards this processes (Bhatia et al., 2005). In the present study, leaf osmolality was increased during Cd and Zn treatment. Similar results were observed in *Stackhousia tryonii* plants exposed to Ni stress (Bhatia et al., 2005). The osmolality of AM plants were less enhanced as compared to non-AM plants because the mycorrhization aid to maintain the tissue moisture content similar as that of

control. But the increase was drastic in non-AM plants. The buildup of osmolality is related to the increase of metabolites such as sugars, amino acids, and proteins. Similarly, soluble sugar, and amino acids content were increased in *Stackhousia tryonii* under Ni toxicity that significantly increased the osmolality of leaves (Bhatia et al., 2005). In AM associated plants, this sort of metabolite accumulation was less on exposure to HMs hence there was only minor increase in osmolality.

In the roots of *O. sativa* and *Z. mays* exposed to Cd and Zn stresses, osmolality was reduced. The moisture content of root tissue increases and correspondingly reduction in osmolality was observed due to the dilution of osmolytes. The reduction in the osmolytes was reflected in the metabolic status in the roots of non-AM and AM plants. However, non-AM roots affected prominent reduction in metabolites under Cd and Zn stresses. The necessity of solute dilution was discussed in the section 5.5. The association with mycorrhization stabilized the water status and accordingly the reduction in the osmolality was less in AM plants.

5.11 Mycorrhizae and heavy metals induced changes in the functional groups of lignin

Lignin is made of three phenyl propane units of *p*-hydroxyphenyl, guaiacyl, and syringyl, with variety of functional groups, including hydroxyls (phenolic and aliphatic hydroxyls), methoxyls, carbonyls, and carboxyls (Zhou *et al.,* 2012). 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} corresponding to the aromatic and aliphatic OH groups, is evident in all the samples isolated from the shoot and roots of *Z. mays* and *O. sativa* plants. Among the different hydroxyl groups, phenolic hydroxyl group indicates the lignin content increase as it aid in 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 of *Z. mays* indicates a lower content of hydroxyl group, denoting that Cd and Zn stresses significantly hindered the polymerization of lignin chain (Thielemans and Wool, 2005; Katahira et al., 2018). Similar results were observed in non-AM plants of *O. sativa* exposed to Zn, wherein polymerization of lignin was hindered as assessed through the reduction in hydroxyl groups. The unaltered peak of Cd treated *O. sativa* plants at 3403 cm^{-1} indicates that there is no significant influence of Cd on the polymerization process of lignin.

The peaks at 1285, 1233, 1178 cm^{-1} are corresponding to the stretching of C-O groups, further these peaks represents aromatic esters, alkyl or aryl ether and esters respectively. These three bands were prominently observed in the roots of *Z. mays* 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 which reduce the permeability of metal ions and thus lowers the metal uptake, protecting the plant from excess metal accumulation (Steudle, 2000). Mycorrhization did not significantly contribute towards the alterations in lignin composition and therefore the metal induced modifications were same in AM and non-AM plants.

The peaks at 2911, 2846, 1716, 1322, 1067, and 849 cm^{-1} are assigned to the symmetrical C–H stretching vibration, asymmetrical C–H stretching vibration, stretching vibrations of C=O bonds in 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} corresponds to C–S stretching indicating the presence of sulphuric acid, which remains as a contaminant in the isolation protocol of lignin samples.

These peaks were common to all the lignin samples isolated from the non mycorrhizal and mycorrhizal plants exposed to Cd and Zn stresses. Therefore, it could be deduced that metal toxicity and mycorrhizal association did not elicit any changes in these specific functional groups of lignin.

5.12 Influence of mycorrhizae on bioaccumulation of Cd and Zn

Earlier, it was reported that mycorrhization improved the metal tolerance of plants by increasing the immobilization of metal ions. In current analysis, mycorrhizae mediated reduction in the Cd accumulaon in the roots of *Z. mays* and *O. sativa* was regarded as an important tolerance mechanism towards metal stress and, similar results were reported in *Triticum aestivum* by Shahabivand et al. (2012) and Sharma et al. (2016). Due to the reduction of Cd accumulation in the roots of AM associated roots, the subsequent translocation of Cd into the leaves was also reduced. 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 pigeon pea, *O. sativa,* and *Z. mays* plants have showed similar tolerance mechanism towards Cd toxicity (Liu et al., 2014; Garg et al., 2015; Rizwan et al., 2018).

Mycorrhization also reduced the uptake of Zn ions to the roots of *Z. mays* and the subsequent translocation of Zn into the *Z. mays* leaves was also reduced as compared to non-AM plants. The reduction in the uptake of Zn due to the mycorrhization was reported by different researchers in various species such as *Medicago truncatula* and *Miscanthus* × *giganteus* plants (Burleigh et al., 2003; Andrejić et al., 2018). At the same time, in *O. sativa* Zn accumulation was increased in the roots of AM plants, but the translocation of Zn ions to the shoot system was restricted.

The higher TF value for the control as compared to the plants subjected to Zn toxicity was due to the low concentration of Zn in the growing media of the control and due to the minimal uptake of the same to the shoot as it is an inevitable essential element in the shoot metabolism. The TF was low in plants exposed to Zn toxicity and mycorrhization further reduced it. The lower TF value under higher Zn concentration indicates the potential of the plant to reduce the translocation of Zn ions to the metabolically more active shoot regions and thus protect the plants from the adverse effects of metal toxicity (Andrejić et al., 2018).

It was reported that under Zn toxicity, the uptake of Zn by plants could be down regulated by mycorrhizal colonization (Burleigh et al., 2003). Moreover, fungal vesicles have the potential to chelate Zn ions and around 300 µg g^{-1} Zn was accumulated in these structures, which was 10 times higher than normally reported in plants (Marques et al., 2007). The AM plants are also able to better handle the HM in the soil by the help of external mycelium which is able to produce glycoprotein glomalin, which have metal binding properties. It was reported that 1g glomalin could extract up to 4.3 mg Cu, 0.008 mg Cd and 1.12 mg Pb from polluted soil (Gonzalez-chavez et al., 2004). The mycorrhizal hyphae also have very important role in phytostabilization by enhancing the passive adsorption of HM to the hyphae which will finally lead to binding up to 0.5 mg Cd per gram dry biomass (Joner et al., 2000; Leyval and Joner, 2001). Immobilization of high Cd and Zn ions in roots may be regarded as the important tolerance mechanism of *Z. mays* and *O. sativa* plants towards metal stress and mycorrhization helps the plant to improve this strategy.

5.13 Influence of mycorrhization on cellular distribution of Cd and Zn

It is important to analyse the pattern of HMs distribution in the roots and leaves and the difference in the localization of metal ions should

contribute to the metal tolerance. Pattern of distribution of Cd and Zn in *Z. mays* and *O. sativa* showed differences. The study conducted in *Bruguiera gymnorrhiza* showed maximum Zn localization in the exodermis and this potential of exodermis to immobilize metal ions is the first line of defence against Zn toxicity (Cheng et al., 2010). These findings could be related to the results obtained in this study, wherein non-AM plants of *Z. mays* and *O. sativa* showed maximum Zn localization in outer regions of the root such as epidermis and cortex. This result was also supported by the findings of Nishizono et al. (1987), wherein they have established that most metals gets localized in the plant cell wall region and the metals complexed with structural materials of the cell wall, such as lignin and cellulose. The lignification observed in the outer region of *Z. mays* and *O. sativa* increased the complexation of these metal ions and hence, relatively decreased the further transfer of excessive Zn to the inner cells or tissues that could aid to reduce the negative impact exerted by the metal to plant. In *Z. mays*, mycorrhization did not significantly influence the pattern of distribution of Zn, but in *O. sativa* this symbiotic association modified the pattern of distribution of Zn. Due to mycorrhization, a shift in the area of localization of Zn from outer region to middle region occured. In contrast to these results, the association with *Rhizophagus intraradices* increase the localization of Zn in the root epidermis of a Ni-hyperaccumulator *Berkheya coddii* (Orłowska et al., 2013).

Along with epidermis, endodermis also strongly prevents the entry of Zn towards the central tissues by immobilizing this metal in the endodermal cell wall (Azzarello et al., 2012). Special affinity of endodermal cells towards Cd was well observed in *Z. mays* roots. Furthermore, mycorrhizal association increases lignification in the cells of endodermis that could be the reason for the overaccumulation of Zn in the endodermal region of *O. sativa*. This was more evidenced by the expression of the transporters responsible for the Zn

accumulation (*OsZIP9*) specifically in the endodermis of root. Parallel results were observed in wheat, the EDXMA analysis performed in the roots exposed to 100 μg g^{-1} Cd^{2+}showed Cd localization in the xylem and endodermis cells (Gao et al., 2015). They proved that the non-thickened outer tangential endodermal wall is the major location of Cd accumulation. In *Z. mays*, the mycorrhizal association did not alter the pattern of distribution of Cd and it was maximum in the endodermis as similar to the non-AM plants.

The role of root periderm and secondary phloem in the adsorption of Cd in cotton was analysed and it was found that Cd deposits were mostly found inside the intercellular space of secondary phloem, in the vessel and near the vascular cylinder of the roots (Chen et al., 2015). Similarly, in non AM plants of *O. sativa*, vascular tissues showed maximum affinity towards Cd and it overaccumulated Cd in leaves. Cadmium was transported into the root xylem via apoplastic and symplastic pathways and the latter was dominant over the former pathway. Therefore, it was clear that the lower concentration of Cd inside the stelar region of *Z. mays* and AM plants of *O. sativa* was due to the prevention of apoplastic movement of Cd to the vascular region (Akhter et al., 2014). The root cell walls form the first line of defence against Cd with the help of negatively charged extracellular sites for metal absorption. Thus, root cell wall of *Z. mays* probably reduces the transport of metals from the root apoplasts to the root xylem (Lasat, 2002). This aid to reduce the translocation of Cd to the leaves, which was reflected in the reduced Cd concentration in the leaves of *Z. mays*. The higher accumulation of Cd in the stelar region of *O. sativa* upregulated its translocation towards leaves, thus it accumulated more Cd in the leaves as compared to *Z. mays*.

In leaves, the palisade tissue of *Phytolacca americana* plants showed maximum Cd localization, and less Cd was found in spongy tissue near the lower epidermis (Fu et al, 2011). In *Picris divaricata* Cd was distributed

mainly in the trichomes, upper and lower epidermis and bundle sheath cells, with a relatively low level of Cd in mesophyll cells (Peng-Jie et al., 2012). In the present study, both non-AM and AM plants of *Z. mays* and *O. sativa* showed significant Cd localization in bundle sheath cells and not in the mesodermis, and this could aid to reduce the impact of Cd on metabolism in leaves. In the case of Zn stress, non-AM plants showed maximum Zn accumulation in mesophyll cells and similar results were found in *Thlaspi caerulescens* (Küpper et al., 1999) and barley (Brune et al., 1994). This could be one of the major reasons responsible for the hypersensitivity of non-AM plants of *O. sativa* towards Zn stress. But, AM plants had different pattern of distribution and the vascular tissues had maximum Zn accumulation.

5.14 Impact of mycorrhization on Cd and Zn induced anatomical modifications

Association of mycorrhiza and HM toxicity elicits different anatomical modifications in *Z. mays* and *O. sativa*. The degradation of parenchymal cells observed in *Z. mays* roots could be the result of oxidative stress induced by the elevated metal concentration in these tissues. Similar results were found in *Brachiaria decumbens* under Cd and lead toxicity, which induced cell wall degradation in roots (Gomes et al., 2011). Vitória et al. (2006) reported that Cd-induced reduction in the cellular hydraulic potential was the reason for the disintegration of the cortical parenchyma of radish roots. This extensive cell degradation significantly reduced the rate of root growth and maturation under heavy metal stress (Sandalio et al., 2001, Shackira and Puthur, 2019). *Oryza sativa* roots did not show any significant parenchymal cell degradation, in contrast to *Z. mays* roots, which indicates lower oxidative stress elicited in *O. sativa* roots as compared to *Z. mays*.

Cell wall thickening is considered as an important anatomical modification in *O. sativa* and *Z. mays* under Cd and Zn stresses. Shackira and

Puthur (2019) reported wall thickening in the root cells of *Acanthus ilicifolius* under Cd stress, and thus supports the results of the present study. This cell wall thickening is largely due to lignification that occurs in response to the presence of high metal concentrations. Cd and Zn-induced lignification in the cell wall has been reported in the roots of different plants, such as *Glycine max* (Finger-Teixeira et al., 2010), tea (Zagoskina et al., 2007), wheat (Li et al., 2012b) and *Z. mays* (da Cunha and do Nascimento, 2009). Heavy metals have the capacity to bind to the cell walls, and the increased cell wall content facilitates this process. The capacity to bind heavy metals in the cell wall is a mechanism to protect cytoplasmic metabolism from the deleterious effects of heavy metals (da Cunha and do Nascimento, 2009). Moreover, the thickening of epidermal, exodermal and endodermal cell walls in *Z. mays* and *O. sativa* roots indicates the adaptation of these plants to elevated concentrations of heavy metals. This thickening also acts as an apoplastic barrier for the uptake and transport of metal ions (Lux et al., 2011). Different ROS molecules accumulated in the roots act as a signaling molecules for lignin deposition in the cell wall, leading to cell wall thickening (Rahoui et al., 2017). Such an arrangement prevents the transportation of Cd and Zn to the shoot, which was prominently observed as Casparian band thickening of the endodermis in the roots of *Z. mays* plants. In addition, when *Z. mays* plants were exposed to Cd stress, the development of Casparian bands and suberin lamellae aided as the apoplasmic barriers for the transfer of Cd content (Vaculík et al., 2012a). Interestingly, in both plants mycorrhizal association increased the epidermal wall thickening that helps to alleviate the Cd and Zn stresses by preventing the entry of these toxic ions to the metabolically active root cells.

Xylem plays a crucial role in water and mineral transport, and metal toxicity induces the deposition of electron-dense compounds in xylary elements, which further reduces the longitudinal transport of water and minerals. In this study, roots of *O. sativa* and *Z. mays* showed metal

deposition in the xylem elements and reduced water transport through xylem vessels. This hindrance due to depositions in the xylary elements in the roots of *O. sativa* and *Z. mays* interfere the water transport to shoots, subsequently hindering plant growth and development. *Phaseolus vulgaris* exposed to Zn stress exhibited less water transport due to Zn deposition in xylem vessels, supporting the results of this study. At the same time, the walls of the xylem sclerenchyma are metabolically inactive, and the cell wall compartmentalization of metals in this area helps the plant to tolerate metal stress. Wall thickening in the xylem elements was comparatively less in AM plants as compared to non-AM plants, and this could be attributed to the reduced metal transportation through this tissue. Interestingly, the cell wall thickening induced by Cd and Zn stresses was different in different tissues of *Z. mays* and *O. sativa*. Cadmium treated roots of *Z. mays* and *O. sativa* showed an increase in wall thickening in endodermal cells, whereas in the roots of Zn-treated roots of *Z. mays* and *O. sativa*, xylem elements showed maximum thickening. This difference could be due to the differential affinity of these cations toward the endodermis and xylem elements (Krzesłowska, 2011).

Cellular injuries are the most common response of leaves toward excess Cd content observed in *Salix viminalis* (Vollenweider et al., 2006). Similar to this, the prominent leaf anatomical modifications induced by Cd in the non-AM plants of *Z. mays* leaves is the degeneration of mesophyll and epidermal cells. But similar response was not observed in the leaves of AM plants due to the effective immobilization of Cd in roots. The vascular bundles of *O. sativa* subjected to Cd stress were smaller, and mesophyll cells containing chloroplasts exhibited chlorosis and air cavities were larger (Tripathi et al., 2012). But in the present study, *O. sativa* leaves did not exhibit similar response and this was due to the high tolerance of *O. sativa* Var. Varsha towards Cd (Sinisha and Puthur, 2018). On exposure to Zn,

Brassica juncea showed breakdown of epidermal, palisade and spongy parenchyma cells (Sridhar et al., 2005). In the present study, leaves of non-AM plants of *Z. mays* and *O. sativa* were more affected due to Zn stress and it was reflected in the structural loss of mesophyll tissues whereas this sort of leaf anatomical modifications were less in AM plants and therefore the metabolic activities of leaves proceeded without much interruption.

5.15 Effect of heavy metal toxicity on mycorrhizal development in host

It is well known that the AM association enhanced phytoremediation potential of plants (Gohre and Paszkowski, 2006). Analysis of AM structural variations during the period of HM treatment showed some changes in developmental features, largely exhibited as the variations of arbuscules and vesicles. The size and number of the vesicles increased with an increase in HM stress. It can be correlated with the report of Kaldorf et al. (1999), wherein they found that heavy metal tolerance in *Z. mays* was due to selective immobilization of HM in fungal tissues like hyphae, arbuscules and vesicles. But on 12 d of exposure, a prominent increase was not observed in the vesicle size but the frequency was increased which indicates simultaneous development of small vesicles along with the old enlarged ones. On close analysis it was observed that the reduction of arbuscular number in control plant was due to degeneration phase in arbuscular developing cycle. But the degeneration of arbuscules was prevented by HM treatment, specifically on Cd treatment. It helps plant to escape from HM toxicity due to the immobilization capacity of arbuscules and it indicates the importance of arbuscules on HM absorption and phytostabilization. Arbuscular cycle is essential to keep the symbiotic relation of AM with the host, but HMs toxicity prevents the dynamics of arbuscular cycle that help the host to escape from HMs stress. This prolonged phase of constancy in arbuscular cycle aid to reduce the impact of metal toxicity in plants.

It was reported that the Zn uptake in plants can be down regulated by mycorrhizal colonization (Burleigh et al., 2003) and it was also supported by the reports of Clark and Zeto (2000). The AM plants are also able to better handle the HM in the soil with the help of external mycelium which is able to produce glycoprotein glomalin, having metal binding properties. Moreover, mycorrhizal plants alleviate the several deleterious effects of metals on plants by sequestration in the AM fungal hyphae (Ryan and Angus, 2003). These hyphae efficiently adsorb metal ions to the cell walls and thus reduce the bioavailabilty of metals to the host (Joner et al., 2000). Increase in the number of spores under Cd and Zn stresses was also an interesting finding of this work. This is an important strategy of the mycorrhiza to survive the metal stress condition, by increasing the rate of multiplication as its spores considered as the better element in the inoculum for colonization with host root.

All the above mentioned properties of AM fungi, indicates that this micro organism exhibits phytostabilization properties, thereby imparts tolerance to the host plants towards HMs such as Cd and Zn. It was proved that heavy metal sensitive plants can be made tolerant by developing association with AM.

5.16 Tolerance index (TI) and mycorrhizal dependency (MD)

Tolerance index was commonly used to estimate plant metal tolerance. For example in the case of *B. juncea* plants, TI was used to measure the tolerance towards various heavy metals (Reisinger et al., 2008). In the case of various other stresses, TI was used to classify different crop varieties as tolerant and sensitive (Koti et al., 2007). The level of Cd stress tolerance of five Indian mustard (*Brassica juncea* L.) cultivars was analysed based on TI, and the results revealed that cultivar Alankar was most tolerant to Cd based on tolerance index (Gill et al., 2011). The reduced TI in Cd exposed *Z. mays*

indicates low tolerance level of this plant. Whereas the tolerance level of *O. sativa* was higher towards the Cd and Zn stresses as represented by the increased TI value. These results could be related to the rapid reduction of growth and photosynthesis with the overaccumulation of ROS molecules observed in *Z. mays* on exposure to Cd and Zn stresses. The mycorrhization potentially improved the tolerance level of *Z. mays* and *O. sativa* on exposure to Cd and Zn stresses. Similar results were observed in *Trigonella* plants exposed to Cd, where AM association increased TI of the plant (Abdelhameed and Metwally, 2019). The better metal stabilization, water absorption, mineral acquisition and photosynthesis facilitated by mycorrhiza aid to improve the tolerance of plants.

In the present study, the impact of mycorrhization was also evaluated with the help of mycorrhizal dependency (MD). It is a common parameter considered for assessing the influence of mycorrhizal association on metal tolerance of plants (Abdelhameed and Metwally, 2019). The results indicate *Z. mays* had higher mycorrhizal dependency than *O. sativa* under Cd and Zn stresses. The low tolerance level of this plant demand higher dependence on mycorrhiza for growth in Cd and Zn contaminated soils. Similar results were observed in *Trigonella* on exposure to Cd stress, where increase in the concentration of Cd caused an increase in MD also. So it was found that the mycorrhizal colonization potentially help to counter the negative impacts of HM in plants, especially in *Z. mays*.

SUMMARY AND CONCLUSIONS

The present study was carried out to investigate the modifications in the heavy metal stress tolerance potential of *Oryza sativa* (variety Varsha) and *Zea mays* (variety CoHM6) having association with mycorrhizae. Various analysis were conducted in the leaves and the roots of *O. sativa* and *Z. mays* to evaluate the functional aspects, metabolic implications and structural remodulations in the plants associated with mycorrhizae and exposed to Cd and Zn stresses.

The extensive ramification of hyphae along with the development of arbuscules and vesicles of *Claroideoglomus claroideum* and *C. etunicatum* in the roots of *O. sativa* and *Z. mays* proved the wide spectrum of host specificity and compatibility of these microbes with the members of monocots. A well-defined arbuscular cycle with the rhythmic formation and degeneration of arbuscules was observed in *O. sativa* and *Z. mays*. Moreover, the quantity of the inocula was found as an important factor determining the rate of colonization, and the increase in the quantity of inocula was reflected as an increase in root cohabitation of *C. claroideum* and *C. etunicatum* with *O. sativa* and *Z. mays*. Forty gram of inoculum, the rhizospheric soil containing spores, vesicles and hyphae of *C. claroideum* was used to establish effective mycorrhizal association, and analyse the mycorrhizae assisted heavy metal tolerance in *O. sativa* and *Z. mays*.

For the selection of 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 analysed for the reduction in moisture content %, total chlorophyll content and an increase in MDA content. Further, 0.45 g Cd kg^{-1} soil and 1.95 g Zn kg^{-1} soil were selected as stress imparting concentrations based on the physio-chemical

responses of *O. sativa* and *Z. mays* to various concentrations of $CdCl_2$ and $ZnSO_4$.

The differential response of these two mycorrhizal fungi in the rate of spore germination, root infection, and protein exudation into the soil aided in screening the tolerance level of these organisms exposed to Cd (0.45 g kg^{-1} soil) and Zn (1.95 g kg^{-1} soil) stresses. In accordance with these results, *C. claroideum* was selected as the heavy metal tolerant species over *C. etunicatum.*

Heavy metal treatment induced inhibition in the growth of *O. sativa* and *Z. mays* was evidenced in the reduction of shoot fresh weight and dry weight, and the reduction in growth was prominent in *Z. mays*. Progressive cell death and impairment in water and mineral uptake under Cd and Zn stresses resulted in an extensive loss of cell viability and proliferation of roots. Association with mycorrhizae efficiently mitigated the negative impact of Cd and Zn stresses on growth of these plants by compensating the root cell death with its extensively ramified fine hyphae. This fungal structure supported the water and mineral acquisition, which improved the growth of *O. sativa* and *Z. mays* in Cd and Zn contaminated soil.

The HM toxicity severely disturbed the water status of the plants by preventing the xylem transport and degenerating root cells, whereas the plants associated with *C. claroideum* increased the water uptake and transport through improved root architecture and restricted metal accumulation in vessels.

Cadmium and Zn toxicity inhibited the photosynthetic machinery, which implicates in the extensive degradation of photosynthetic pigments and the functional impairment of the PSII activity. The impact on photosynthesis

further increased due to the HMs induced reduction in the efficiency of electron transport and water-splitting complex. Moreover, Cd and Zn severely restricted the stomatal opening that causes reduction in the gaseous exchange, and the impact on stoma was prominently observed in *Z. mays*. The circular-shaped special structures seen as an opening on the leaf surface of non-mycorrhizal plants exposed to Zn stress, possibly was a means for the emission of volatile compounds, zinc phosphates and zinc sulphates synthesized as a result of excess Zn ion in cell, which can lead to the reduced rate of photosynthesis in *O. sativa* exposed to Zn stress. The absence of the circular-shaped special structures in the leaf surface of the AM plants of *O. sativa* indicates better prevention in the metal uptake and balanced water status of the cell that support photosynthesis. Mycorrhization maintained the photosynthetic efficiency of *O. sativa* and *Z. mays* by improving the pigment content, functionality of PSII, efficiency of electron transport, and by preventing stomatal closure even under the extreme metal stress. Moreover, of the different JIP parameters; area, F_V/F_O, $F_V/F_{M,}$ and RC/CS_M showed significant changes and could be used to assess the intensity of stress as well as the tolerance potential of mycorrhizae associated *O. sativa* and *Z. mays* under Cd and Zn toxicity.

The cellular ROS dynamism and homeostatic events were severely affected due to the high Cd and Zn content in cytoplasm. The oxidative burst due to imbalance in ROS dynamism leads to the membrane degradation and MDA accumulation in *O. sativa* and *Z. mays*. The damage in cellular membrane is reflected in the increase of electrolyte leakage and it directs to the extreme loss in membrane stability. Mycorrhization mitigated the oxidative stress by preventing the overaccumulation of ROS molecules, membrane degradation, and electrolyte leakage in root and shoots of *O. sativa* and *Z. mays* on exposure to Cd and Zn toxicity.

Exposure to heavy metals caused significant alterations in the metabolic harmony of *O. sativa* and *Z. mays* and elicited the overproduction of various metabolites such as protein, sugar, amino acids, and proline at the earlier stages of stress. Phenolics, flavanoids, anthocyanins, alkaloids, ascorbate and glutathione were also increased in shoots. All these metabolites could counter the oxidative stress due to the metal toxicity by increasing the antioxidant activity and metal chelation efficiency of plants. On 4 d of stress, a drastic increase in these metabolites due to the ROS outbreak and oxidative stress was observed in non-AM plants, but AM plants maintained the metabolic status similar as that of control where the impact of oxidative stress was minimum due to effective water uptake and reduced metal translocation. But at the later stages of HM exposure (12 d), the metabolites were decreased in non-AM plants indicating extreme inhibition in the metabolic processes but the reduction was lower in AM plants. Among different metabolites, response of anthocyanin in both plants was different towards Cd and Zn stresses. Moreover, the drastic increase of anthocyanin content in plants subjected to Zn stress recommends this pigment molecule as a potential indicator for the increased bioaccumulation of Zn.

Cadmium and Zn toxicity caused a reduction in the protein, sugar, amino acids, phenolics, flavanoids, proline, anthocyanins, alkaloids, ascorbate and glutathione contents in the roots of *O. sativa* and *Z. mays*. It indicates the impact of HM stress significantly amended the metabolic partitioning between shoot and roots of the plants, which cause overaccumulation of metabolites in shoot with a reduction of the same in roots. The reduction of these metabolites content has strong relation with the enhancement in the tissue damage in roots by the oxidative stress elicited by Cd and Zn ions. The extreme reduction in root metabolic contents of non-AM plants reflects the prominent oxidative damages in the root tissues. The crucial role of

mycorrhiza in metal stress tolerance is indebted to the improved metabolic status in roots under Cd and Zn stresses. In the case of AM plants, the intensity of oxidative stress was less and this symbiotic association adied in maintenance of the metabolic status of the root as similar as control by the effective stabilization of metal ions in the rhizosphere that cause less interference in the metabolism.

Heavy metal toxicity reflects in the dynamism of the bioactive compounds found in *O. sativa* and *Z. mays*. Metal induced the accumulation of potent antioxidant molecules such as gamma-sitosterol, 3-beta-acetoxystigmasta-4,6,22-triene and cholesta-4,6-dien-3-ol, benzoate in these plants. Moreover, Cd and Zn stressors altered the ratio between phytol (RT-27.387) and (E)-phytol (RT-31.849) in both mycorrhizal and non-mycorrhizal plants indicating the stress induced accumulation of fatty acid phytol esters or the conversion of one form of phytol to another. The results specified that in *O. sativa,* mycorrhization influence the ratio between these two forms of phytols. However, in the case of *Z. mays,* the ratio between phytol and (E)-phytol was independent to the mycorrhizal association due to the extreme stress experienced in this plant. More interestingly, it was found that, the alteration in the ratio between phytol and (E)-phytol was based on the type of treated metal ions. The ratio between phytol and (E)-phytol increased under Cd toxicity, but it was decreased on exposure to Zn stress.

Mycorrhization induced the exudation of different bioactive compounds from the roots of *O. sativa* and *Z. Mays*, which potentially help to detoxify the toxic metal ions in the rhizosphere. The exudation of tributyl acetyl citrate, 3-beta-acetoxystigmasta-4,6,22-triene and linoleic acid from the mycorrhizal roots of *O. sativa* and the elicitation of 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 *Z.*

mays was considered as a strategy of mycorrhizal plants to tolerate the extreme stress, as these compounds have crucial roles in the stabilization of metal ions.

The oxidative stress elicited in the cells accelerated the activity of enzymatic antioxidants such as SOD, CAT, APX, and GPOX. Enhanced activity of these biocatalysts in the non-AM plants of *O. sativa* and *Z. mays* at initial stages of the stress indicates that a well-defined antioxidant machinery is operational under heavy metal stress in order to detoxify the ROS. But, exposure to an extreme high level of metal stress damaged the antioxidant machinery of these plants, and prominent stress impact was observed in *Z. mays*. Mycorrhization partially offset the negative impact of Cd and Zn stresses and maintain the increased activity of all the four enzymes even in the later stages of stress, whereas non-AM plants failed to maintain the enzyme activity at the later stages of stress and this could be due to the excess degradation of proteins.

Bioaccumulation pattern of Zn and Cd revealed that the most of the metals taken up by the plants are accumulated in the root itself rather than translocating to the aerial parts of the plant. Both *O. sativa* and *Z. mays* executes this key character of phytostabilizer with a low TF<1 (0.1-0.7), indicating the effective metal tolerance mechanism of these plants. Mycorrhization assists to increase the kinetics of metals (Cd and Zn) immobilization by binding with the glycoprotein glomalin released by AM fungi. Moreover, different structures of AM such as hyphae, arbuscules, and vesicles potentially stabilize toxic metals by adsorbing the metal ions in the cell wall of the hyphae.

Differential distribution of Cd and Zn in the root is considered as an excellent strategy to overcome the random flow of these toxic metals towards metabolically active sites. Epidermis and endodermis potentially acts as

barriers for the transfer of metal ions and the inactive elements of xylem plays an important role in the sequestration of Cd and Zn ions.

Cadmium and Zn induced anatomical modifications were visualized by the microscopical characterization of root and leaf of *O. sativa* and *Z. mays*. The impact of Cd and Zn stresses on the anatomical features were prominent in the latter plant. Cortical cell degradation, crystal formation, and cell wall thickening were the major anatomical responses observed in *O. sativa* and *Z. mays* towards HM stress. The crystal formation inside the vessels prominently hinders water transport through xylem resulting in the reduction of moisture content in leaves. Even though, the mycorrizal plants exposed to Cd and Zn stresses increased the cell wall thickening and crystal formation in vessels, the intensity of cell degradation was minimized in the roots. Due to the efficient prevention of metal ions to the leaves, the anatomical modifications were insignificant in the leaves of mycorrhizal plants as compared to non-AM plants.

Cell wall thickening is one of the major strategies of these plants to sequestrate more metal ions in the inactive part of the cell. One of the major element of cell wall *i.e.,* lignin when characterized, prominent variations in functional groups were observed corresponding to the peaks at 3403, 1285, 1233, 1178 cm^{-1}, that represents the improved esterification in lignin.

The inhibition observed in the arbuscular cycle, and increases in the frequency of the vesicles were the major structural changes in AM fungi on exposure to HMs stresses, and it efficiently enhances the stabilization process of Cd and Zn. All the results make it clear that *O. sativa* was the most tolerant plant towards Cd and Zn stresses as compared to *Z. mays* due to the better maintenance of photosynthetic efficiency, fresh weight, moisture content, anatomy and root metabolic status which was reflected in the tolerance index. The difference in the mycorrhizal dependency of *Z. mays*

and *O. sativa* is a crucial factor, and the influence of mycorrhizal colonization on HM stress tolerance of *Z. mays* was prominent as compared to *O. sativa.*

The present study evidenced the efficiency of mycorrhization to ameliorate the adverse effects of Cd and Zn stresses in *Z. mays* and *O. sativa*, by sequestering toxic metal ions in AM fungal structures, mitigating the oxidative stress, maintaining the metabolic status, boosting the antioxidant activity, and altering the bioaccumulation pattern of the metals in the hosts.

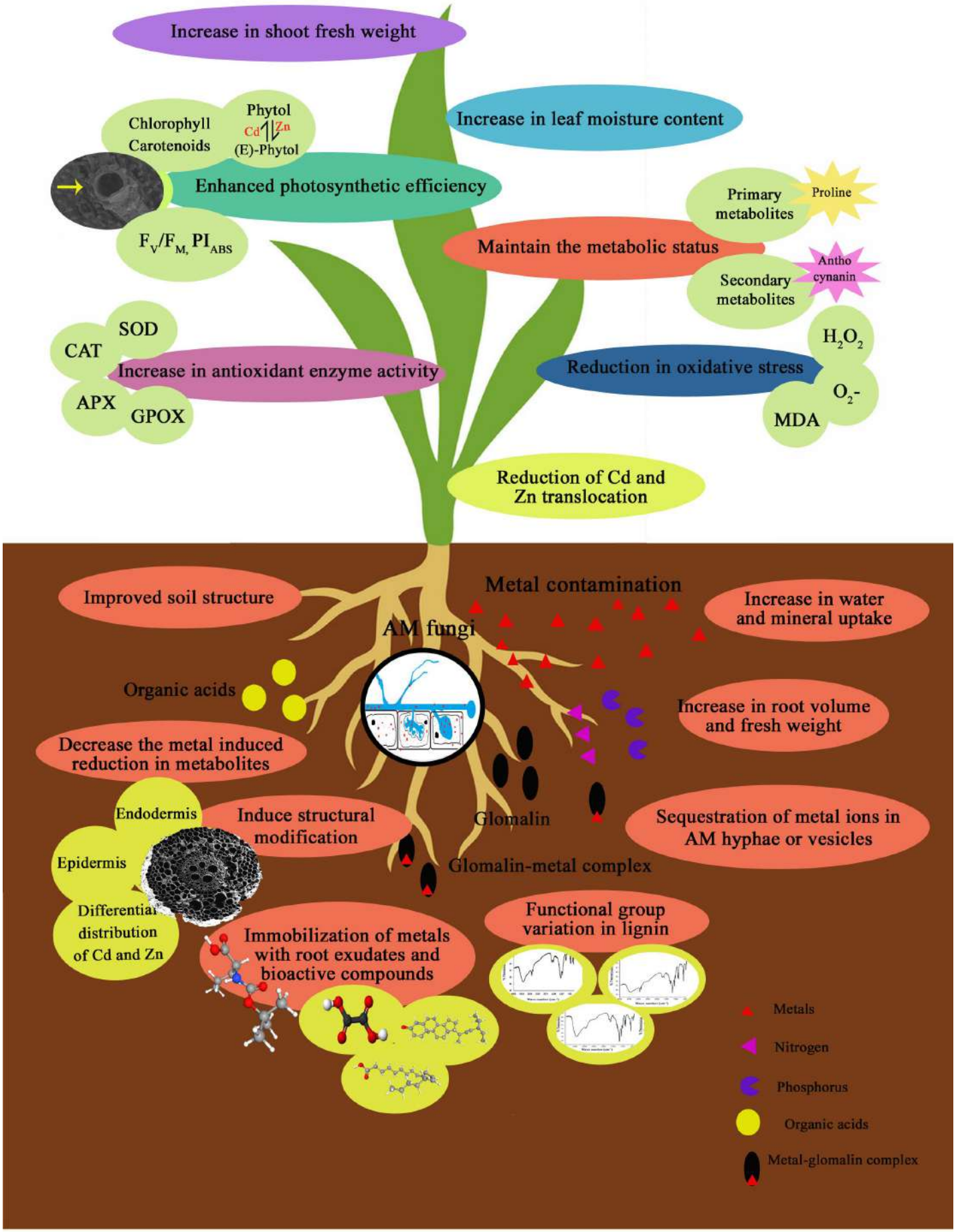

Figure 86: Mycorrhizae mediated enhanced heavy metals (Cd and Zn) stress tolerance in *O. sativa* and *Z. mays*.

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LIST OF PUBLICATIONS AND PRESENTATIONS

Publications in research journals

1. **Janeeshma, E.,** & Puthur, J. T. (2020). Direct and indirect influence of arbuscular mycorrhizae on enhancing metal tolerance of plants. *Archives of Microbiology*, *202*(1), 1-16. (IF 2.552)

2. **Janeeshma, E.,** Puthur, J. T., & Ahmad, P. (2020). Silicon distribution in leaves and roots of rice and maize in response to cadmium and zinc toxicity and the associated histological variations. *Physiologia Plantarum*. 10.1111/ppl.13310 (IF 4.5)

3. **Janeeshma, E.,** Kalaji, H. M., & Puthur, J. T. (2021). Differential responses in the photosynthetic efficiency of *Oryza sativa* and *Zea mays* on exposure to Cd and Zn toxicity. *Acta Physiologiae Plantarum*, *43*(1), 1-16. (IF 2.354)

4. **Janeeshma, E.,** Rajan, V. K., & Puthur, J. T. (2021). Spectral variations associated with anthocyanin accumulation; an apt tool to evaluate zinc stress in *Zea mays* L. *Chemistry and Ecology*, *37*(1), 32-49. (IF 2.244)

5. Arun, K., **Janeeshma, E.,** Job, J., & Puthur, J. T. (2021). Physiochemical responses in coconut leaves infected by spiraling whitefly and the associated sooty mold formation. *Acta Physiologiae Plantarum*, *43*(3), 1-13. (IF 2.354)

6. Hiba, H., **Janeeshma, E.,** & Puthur, J. T. (2021). Dynamic alterations of metabolites in *Plectranthus amboinicus* (Lour.) Spreng. to encounter drought and Zn toxicity. *Brazilian Journal of Botany*. (Accepted) (IF 1.296)

7. **Janeeshma, E.,** & Puthur, J. T. (2021). Physiological and metabolic dynamism in mycorrhizal associated *Oryza sativa* (var. Varsha) towards Zn and Cd toxicity Environmental science and pollution research. *Environmental Science and Pollution Research.* (Communicated)

8. **Janeeshma, E.**, Wróbel, J., Kalaji H. M., & Puthur, J. T. (2021). Metabolic alterations elicited by Cd and Zn toxicity in *Zea mays* with the association of *Claroideoglomus claroideum*. *Ecotoxicology*. (Communicated)

Book chapter

1) **Janeeshma, E.,** & Puthur, J. T. (2018). Potential role of microbial endophytes in xenobiotic stress management. In: Virendra Mishra Ajay Kumar (eds), *Sustainable Environmenta lean-up green remediation*. Elsevier Netherlands (ISBN 9780128238288), pp-165-185.

2) **Janeeshma E.,** Gurudatta Singh, Jos T. Puthur (2021) Microbial mediated waste management and bioenergy production, In: Utilization of waste biomass in energy, environment and bio-catalyst. Taylor & Francis/CRC Press (In Press)

3) **Janeeshma E.,** Akhila Sen, K. P. Raj Aswathi, Riya Johnson and Jos T. Puthur* Reclamation and phytoremediation of heavy metal contaminated land "Bioenergy crops: a sustainable means of phytoremediation" (In Press).

4) **Janeeshma, E.,** Sameena P.P, Sarath G. Nair, Veena mathew and Jos T. Puthur Impact of pesticide on the soil microbiology. In: Pesticides in Natural Environment: Sources, Health Risks, and Remediation" Elsevier, USA (In press)

Papers in seminars/conference proceedings

1) **Janeeshma, E.,** & Puthur, J. T. (2020). Phytochemical analysis of *Zea mays* associated with arbuscular mycorrhizae and subjected to cadmium and zinc stresses. International webinar "DOCTRINA -11" on “Plant Functional Biology”. Department of Botany, Sir Syed college, Taliparamba, Kerala.

2) **Janeeshma, E.,** & Puthur, J. T. (2019). Histochemical changes in roots and leaves of maize seedlings subjected to cadmium stress. “PROVECTUS PLANTAE'19”. International conference on ‘exploring the scope of plant genetic resources’. Department of Botany, University of Kerala. ISBN: 978-81-940888-0-6. 2365-2676.

3) **Janeeshma, E.,** Mirshad, P. P., & Puthur, J. T. (2016). Dynamics of arbuscular mycorrhizal development in relation to various growth stages of *Zea mays* L. In *28th Kerala Science Congress (Jan 2016) Extended Abstracts* (pp. 1975-1979). Proc. 28th Kerala Science Congress, 243-251.

Presentations in national/international seminars/conferences:

1) **Janeeshma, E.,** & Puthur, J. T. (2020). Phytochemical analysis of *Zea mays* associated with arbuscular mycorrhizae and subjected to cadmium and zinc stresses. International webinar on plant functional biology organized by Department of Botany, Sir Syed College, Thaliparamba, Kannur, June 05 - 07, 2020.

2) **Janeeshma, E.,** & Puthur, J. T. (2019) Histochemical changes in roots and leaves of maize seedlings subjected to cadmium stress, at International conference on exploring the scope of plant genetic resources, organized by Department of Botany, University of Kerala, May 22-24, 2019.

3) **Janeeshma, E.,** & Puthur, J. T. (2019) Responses of Zea mays and Oryza sativa to different concentrations of cadmium; a comparative study. National Conference of Plant Physiology on “Plant Productivity and Stress Management”, organized by Kerala Agricultural University, Thrissur and Indian Society for Plant Physiology, New Delhi, December 19-21, 2019.

4) **Janeeshma, E.,** & Puthur, J. T. (2019) Root physiology and phytochemistry related to cadmium toxicity in rice associated with arbuscular mycorrhizae, at XLII all India botanical conference of the Indian botanical society and national symposium, on "Innovations and Inventions in Plant Science Research", organized by Department of Botany, University of Calicut, November 06-08, 2019.

5) **Janeeshma, E.,** & Puthur, J. T. (2018) Anatomical and pigment variations in Zea mays under different concentrations of zinc. National seminar on ' Recent trends in climate change and carbon research' at Department of Botany, University of Calicut, Malappuram, February 26-28, 2018.

6) **Janeeshma, E.,** & Puthur, J. T. (2018) Zinc induced variations in metabolites accumulated in *Oryza sativa* as influenced by mycorrhizal association. National seminar on climatic vagaries and biodiversity issues contemporary and futuristic, Department of Botany, SN College, Kannur. September 17-18, 2018.