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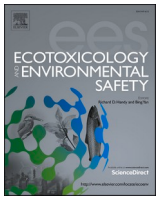

Iron nanoparticles in combination with other conventional Fe sources remediate mercury toxicity-affected plants and soils by nutrient accumulation in bamboo species

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ABSTRACT

The issue of mercury (Hg) toxicity has recently been identified as a significant environmental concern, with the potential to impede plant growth in forested and agricultural areas. Conversely, recent reports have indicated that Fe, may play a role in alleviating HM toxicity in plants. Therefore, this study's objective is to examine the potential of iron nanoparticles (Fe NPs) and various sources of Fe, particularly iron sulfate (Fe SO_4 or Fe S) and iron-ethylene diamine tetra acetic acid (Fe - EDTA or Fe C), either individually or in combination, to mitigate the toxic effects of Hg on *Pleioblastus pygmaeus*. Involved mechanisms in the reduction of Hg toxicity in one-year bamboo species by Fe NPs, and by various Fe sources were introduced by a controlled greenhouse experiment. While 80 mg/L Hg significantly reduced plant growth and biomass (shoot dry weight (36%), root dry weight (31%), and shoot length (31%) and plant tolerance (34%) in comparison with control treatments, 60 mg/L Fe NPs and conventional sources of Fe increased proline accumulation (32%), antioxidant metabolism (21%), polyamines (114%), photosynthetic pigments (59%), as well as root dry weight (25%), and shoot dry weight (22%), and shoot length (22%). Fe NPs, Fe S, and Fe C in plant systems substantially enhanced tolerance to Hg toxicity (23%). This improvement was attributed to increased leaf-relative water content (39%), enhanced nutrient availability (50%), improved antioxidant capacity (34%), and reduced Hg translocation (6%) and accumulation (31%) in plant organs. Applying Fe NPs alone or in conjunction with a mixture of Fe C and Fe S can most efficiently improve bamboo plants' tolerance to Hg toxicity. The highest efficiency in increasing biochemical and physiological indexes under Hg, was related to the treatments of Fe NPs as well as Fe NPs + FeS + FeC. Thus, Fe NPs and other Fe sources might be effective options to remove toxicity from plants and soil. The future perspective may help establish mechanisms to regulate environmental toxicity and human health progressions.

1. Introduction

Heavy metal (HM)s exist in nature are typically essential for life (Emamverdian et al., 2024). Nevertheless, the accumulation of excessive HM quantities in the soil can give rise to significant environmental and human health concerns (Emamverdian et al., 2024; Agnihotri and Seth, 2019). Mercury (Hg) has become a global environmental contaminant (Ma et al., 2022). It was observed that Hg can remain in the air for a longer period than other HM contaminants, which contributes to air pollution on a global scale through monsoon circulation (Ma et al., 2022). Mercury (Hg) is a naturally occurring element, but it is also a byproduct of human activities. Natural sources of Hg include rock

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weathering and volcanic activities. Anthropogenic sources include Hg and gold mining, cement production, nonferrous metal smelting, and fossil fuel combustion (Du et al., 2019). Annual Hg emissions from human activities in China range from 500 to 1000 tons (Obrist et al., 2018). These substances enter forest soils in the form of dry deposition or litter deposition. That constitutes 70% of Hg input flux to Southwest China forestland (Zhou et al., 2013, 2015). In this case, the Hg deposits in soil organic matter and is maintained in forest soils (Gong et al., 2014; Pokharel and Pokharel and Obrist, 2011). Furthermore, organic soil layers on the upper side are able to retain 95% of Hg (Juillerat et al., 2012). In China, the primary source of Hg on the soil surface is atmospheric Hg deposition (Zhang et al., 2020) due to the country's significant air pollution. The mean Hg concentration in agricultural soil can reach 0.108 mg/kg (Zhong et al., 2016). Hg levels in the farming soil of Shanghai, Zhejiang, Guangdong, Jiangxi, Liaoning, Hubei, and Yunnan vary from 0.100 mg/kg to 0.200 mg/kg. The average concentration in Southern China is higher than in the Northern region (Wang et al., 2016). This might result in soil contamination and continue to be a risk to human life through plant absorption. Leaves in forest trees absorb atmospheric Hg as reactive gaseous Hg, particulate Hg, and gaseous element Hg (Gong et al., 2014). The elevated Hg concentrations in the soil were found to have detrimental impacts on several aspects of plant growth and development. These effects include decreased chlorophyll synthesis, impaired water absorption, reduced transpiration, diminished photosynthetic efficiency, and increased membrane lipid peroxidation (Gworek et al., 2020). Plant cells naturally produce reactive oxygen species (ROS), via a regular metabolic process. However, metal stress induce an excess of ROS production, which can result in the breakdown of proteins and enzymes, as well as the leakage of electrolytes from the cell membranes. Furthermore, ROS can disrupt the structure of cell pigments, ultimately leading to the in vivo death of the plant cell (Hasanuzzaman et al., 2020; Ramakrishnan et al., 2022; Emamverdian et al., 2020). ROS compounds consist of non - radical molecules, including hydrogen peroxide (H_2O_2), singlet oxygen ($1\ O_2$) as well as free radicals such as hydroxyl radical (•OH) and superoxide anion ($O_2^{\bullet -}$) (Sharma et al., 2012)·H_2O_2 was demonstrated to reduce photosynthetic pigments, plant growth and yield, and leaf relative water content (RWC). Additionally, H_2O_2 was shown to damage plant cellular membranes (Choudhary et al., 2024). Plants develop multiple defense systems to counteract environmental stressors. These involve the stimulation of antioxidant enzymes like superoxide dismutase (SOD), peroxidase (POD), glutathione reductase (GR), and catalase (CAT), which play a role in removing reactive oxygen species (ROS) and protecting plant cells (Sytar et al., 2013). The regulation of antioxidant activities enhances plant resistance to abiotic stress when Fe is applied exogenously. This can lead to a reduction of ion toxicity while preserving the balance of nutrients in the soil solution (Ashraf et al., 2023). Numerous antioxidant enzymes and chlorophyll comprise Fe, an essential element (Yang et al., 2023; Nasar et al., 2022). Plants with reduced Fe concentrations had low levels of ferrochelatase, ribonucleotide reductase, and nitrogenase activity, which affects chlorophyll production, the DNA replication repair and, nitrogen fixation in the rhizosphere (Liu et al., 2022; Carter et al., 2022). Adding iron sulfate (Fe SO_4) and iron chelates (Fe - EDTA) to soils with iron deficiencies, regardless of whether they are normal or polluted, is a widely used agricultural method (Singh and Singh, 2022). However, the Fe recovery from Fe SO_4 (19–20.5% of Fe) and Fe - EDTA (9–12% of Fe) is too low (Xia et al., 2022). Hasan et al. (2020) and Jiang et al. (2021) recently documented an increase in the efficacy of nanoparticle-based micronutrients under stress conditions, which can affect plant antioxidant systems and biochemical enzymes (Verma et al., 2023). Moreover, Fe as an inorganic metal nanomaterial (Kumar et al., 2024) may be an optimal nanoparticle for mitigating HM toxicity in plants, according to recent evidence (Javad et al., 2023; Emamverdian et al., 2023; Mariyam et al., 2024). This is due to the Fe NPs' high surface area-to-weight ratio. Under adverse conditions, the ameliorative effect of Fe NPs has also been reported on several plant species, including soybean (Yang et al., 2020), wheat (Merinero et al., 2022), and strawberry (Mozafari et al., 2018a). Fe NPs displaying high reactivity in their surface have the potential to serve as favorable sites for the absorption and the immobilization of HM ions (Zia-ur-Rehman et al., 2023a). In the soil, the oxidation of the Fe NPs results in the release of proton ions (H^+), which reduces the soil pH. This increases the plants' access to essential nutrients such as phosphorus and zinc (Yoon et al., 2019; Ghassemi-Golezani, and Abdoli, 2021). Consequently, the use of Fe and Fe sources represents a proposed solution for the removal of metal toxicity and the enhancement of plant defense mechanisms in species.

In this study, we investigated the potential of using Fe NPs, Fe sulphate (Fe SO_4), and Fe chelates (Fe - EDTA) to mitigate Hg toxicity in plants and soil. "Fe can contribute to enhancing plant resistance" is the proposed hypothesis in this work. Although limited in number, several studies have reported that the presence of Fe and Fe sources can potentially alleviate salt stress (Zia-ur-Rehman et al., 2023a) and HM toxicity (Zhang and Zhang, 2020). To the best of our knowledge, this is the first research into how Fe and different Fe sources can lower Hg levels in bamboo species. Our hypothesis is that the presence of Fe NPs and conventional Fe sources can mitigate the damaging impact of Hg in bamboo species through specific mechanisms. This might be a crucial advancement in the investigation of Fe NPs and other Fe sources for environmental detoxification and enhancing the resistance of bamboo plants.

Bamboo, belonging to the *Bambusoideae* subfamily, exhibits rapid growth and a substantial biomass. It is prevalent in the vast forested areas of Asia, especially in the Southern and Southeastern China (Ahmad et al., 2021; Emamverdian et al., 2018, 2021b). According to Bian et al. (2019), bamboo species have been utilized as a phytoremediation method due to their high biomass and rapid vegetative development. These plants also possess a remarkable capacity for ion extraction through their roots. Mdel et al. (2013) demonstrated that ornamental plants can be used as an indicator of pollution. They serve as a way to evaluate the plant's ability to withstand metal stress in urban areas and potentially play a key role in reducing toxicity. In addition to their aesthetic value, ornamental plants may be utilized to clean up contaminated sewage and polluted air in metropolitan areas when grown for profit (gardening/beautification)(Liu et al., 2008). One type of decorative evergreen bamboo, *Pleioblastus pygmaeus*, was brought to China from Japan in the early 20th century. This species is cabaple of thriving in a wide range of soil PH (acidic, alkaline, or neutral soils) and grows to an average height of 30–50 cm (Huang et al., 2020). This species has expanded not only throughout Jiangsu but also throughout most of China. However, a recent estimate suggests that there are approximately 4.1×10^{4} metric tons of Hg in China's forested and farmed areas (Zhou et al., 2018). The probable Hg existence in bamboo species poses a threat to human health and has the potential to impede the growth of bamboo plants in the area (Zhou et al., 2018). Hence, in order to mitigate and decrease the harmful effects in agricultural and forest areas, as well as the surrounding ecosystem, it is crucial to identify and develop targeted and suitable methods and bio - nutrient components. This paper presents a novel approach to reducing Hg levels in bamboo species that has not been previously documented. The approach employs Fe NPs and other traditional Fe sources. The current research novelty and objective of this work are related to clarifying the process and investigating how Fe NPs and different Fe sources (such as iron sulfate, Fe SO_4, and iron-ethylene diamine tetraacetic acid; Fe - EDTA), both in separated and combined forms, might improve bamboo plants' tolerance to Hg toxicity.

2. Materials and methods

2.1. Plant growth and experimental design

This experiment used a controlled greenhouse with a photoperiod of

16 h of light, 8 h of darkness, a relative humidity of 69–79%, and 18 °C and 24 °C, with vapor pressure deficit (VPD) of 0.5–0.8 kPa. The shading of greenhouses by means of the application of a 20% green roof was implemented with the objective of regulating the intensity of solar radiation.The bamboo species *Pleioblastus pygmaeus* was obtained from the Bamboo Research Institute at Nanjing Forestry University in Jiangsu Province, China. For plant cultivation, 30 * 30 cm pots were used. Each pot included five bamboo plants. The growing media consisted of a mixture of perlite and coco peat, with a ratio of 1:2, in a 3 L volume. The experiment design included: 80 mg/L of Hg alone, in combination with 60 mg/L of Fe NPs, 60 mg/L of Fe SO_4, 60 mg/L of Fe -EDTA, 30 mg/L of Fe NPs + 30 mg/L of Fe SO_4, 30 mg/L of Fe NPs + 30 mg/L of Fe - EDTA, 30 mg/L of Fe SO_4+ 30 mg/L of Fe - EDTA, 20 mg/L of Fe NPs + 20 mg/L of Fe SO_4+ 20 mg/L of Fe - EDTA (Suppl Table 1). All of them were irrigated five times in four replicates for a total solution volume of 250 mL throughout the study in 60 days. Fertilizers for bamboo plants included 400 mL of nutrient solution every five days (Emamverdian et al., 2023), P_2O_5 (calcium superphosphate), ammonium sulfate (nitrogen fertilizer), and potassium sulfate (K_2O). The photosynthetic and morphological characteristics were assessed inside a controlled greenhouse. The harvested bamboo samples comprising stems, leaves, and roots were transferred to the lab at the end of the experiments. For the nutritional and physiological analysis, samples were maintained in a Haier - China refrigerator at −20 °C to analyze metabolic and biochemical parameters.

Table 1
The Impact of 60 mg L^{-1} Fe NPs, Fe C, and Fe S individually or in combination on chlorophyll pigments (Chl-a, Chl-b, Total Chl, and Carotenoid) in bamboo plants (*Pleioblastus pygmaeus*) under 80 mg L^{-1} Hg. The data indicate the mean ± standard error of four replicates. The treatments include different Fe NPs, Fe S, and Fe C levels individually or in combination under 80 mg L^{-1} Hg. The lowercase letters (a, b, c, d, etc.) display significant differences among treatments. Tukey, ($p < 0.05$).

Treatment	Chl-a (mg g^{-1} F.w.)	Chl-b (mg g^{-1} F. w.)	Chl a+b (mg g^{-1} F.w.)	Caratenoids (mg g^{-1} F. w.)
control	4.75± 0.07^{a}	5.66 ± 0.06^{a}	10.43 ± 0.12^{a}	2.33 ± 0.04^{a}
60 mg L^{-1} FeNPs	6.21 ± 0.07 ^{f}	8.36 ± 0.06 ^{g}	14.57± 0.12^{e}	3.42 ± 0.04^{e}
60 mg L^{-1} FeS	5.43± 0.07 ^{cd}	6.78 ± 0.06^{d}	12.21± 0.12^{c}	2.97 ± 0.04^{bc}
60 mg L^{-1} FeC	4.97± 0.07^{ab}	5.98 ± 0.06^{b}	10.95. ± 0.12^{a}	2.51± 0.04^{a}
60 mg L^{-1} FeNPs+FeS	5.89 ± 0.07^{ef}	7.45 ± 0.06^{e}	13.34± 0.12^{d}	3.21 ± 0.04^{de}
60 mg L^{-1} FeNPs+FeC	5.67± 0.07^{de}	7.05 ± 0.06^{d}	12.72± 0.12^{c}	3.12 ± 0.04 ^{cd}
60 mg L^{-1} FeS+FeC	5.17± 0.07^{bc}	6.44 ± 0.06^{c}	11.61± 0.12^{b}	2.77± 0.04^{b}
60 mg L^{-1} FeNPs+FeS+FeC	6.07 ± 0.07 ^{f}	7.96 ± 0.06 ^{f}	14.03± 0.12^{e}	3.33 ± 0.04^{de}
80 mg L-1 Hg	1.11 ± 0.05^{a}	3.44± 0.05^{a}	5.56± 0.11^{a}	0.82 ± 0.04^{a}
60 mg L^{-1} FeNPs+80 mg L^{-1} Hg	4.44± 0.05 ^{f}	5.23± 0.05 ^{f}	9.67± 0.11 ^{g}	2.21 ± 0.04 ^{f}
60 mg L^{-1} FeS+80 mg L^{-1} Hg	3.15 ± 0.05^{c}	4.12 ± 0.05^{c}	7.27± 0.11^{d}	1.45 ± 0.04^{c}
60 mg L^{-1} FeC+80 mg L^{-1} Hg	2.45± 0.05^{b}	3.65 ± 0.05^{ab}	6.01 ± 0.11^{b}	1.03± 0.04^{b}
60 mg L^{-1} FeNPs+FeS +80 mg L^{-1} Hg	3.89± 0.05^{e}	4.67 ± 0.05^{de}	8.56± 0.11 ^{f}	1.92 ± 0.04^{de}
60 mg L^{-1} FeNPs+FeC +80 mg L^{-1} Hg	3.45± 0.05^{d}	4.45 ± 0.05^{d}	7.9± 0.11^{e}	1.76 ± 0.04^{d}
60 mg L^{-1} FeS+FeC +80 mg L^{-1} Hg	2.88± 0.05^{c}	3.87± 0.05^{bc}	6.75± 0.11^{c}	1.29 ± 0.04^{c}
60 mg L^{-1} FeNPs+FeS+FeC+80 mg L^{-1} Hg	4.14± 0.05^{e}	4.89 ± 0.05^{e}	9.03± 0.11 ^{f}	2.04 ± 0.04^{ef}

Fe-NPs were acquired from Nanjing Jiancheng Company, Nanjing, Jiangsu, China, in powder form with a particle size range of 55–100 nm and 97% purity. The powder's surface area was 19–51 m^2/g, density was 5.1 g/m^3, and it was different in particle size from other typical Fe sources (Fe SO_4+ Fe - EDTA).

2.2. Nutrients and Hg accumulation

0.5 g dry leaf samples incubated in 5 mL nitric acid at 30 °C overnight. The mixture was dried in an oven (China Energy) set at 95 °C. The nutritional concentration, including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), as well as the Hg levels, were assessed using the methodology of Ismail et al. (2022) which employed mass spectrometry (MS) combined with inductively coupled plasma (ICP) for analysis (Agilent 4500 series, USA) (Khosropour et al., 2019).

Cell membrane stability assessment and photosynthetic pigments. The membrane stability index (MSI) was determined by method of Premachandra et al. (1990) and Sairam (1994). Twenty mL of distilled water was present with bamboo leaves in test tubes in order to determine the Electrical Conductivity (EC_1). The test tubes were subsequently moved to an autoclave, and once the samples cooled down, Electrical Conductivity (EC_2) were analyzed for MSI calculation. The formula below was applied to compute the MSI.

$$MSI = (1- (EC_1/EC_2)) \times 100 \quad (1)$$

The leaf relative water content (LRWC) was determined using a modified equation based on the procedure established by Barrs and Weatherley (1962). The calculation of LRWC was performed as:

$$LRWC\ (\%) = (FW - DW) / (TW - DW) \times 100 \quad (2)$$

where fresh weight (FW), turgid weight (TW), and dry weight (DW) were summarized.

According to the method of Lichtenthaler and Wellburn (1985), chlorophyll a, b, and carotenoids were determined by measuring absorbances at 663 nm, 645 nm, and 470 nm. The data was reported as mg g^{-1} fresh weight (FW).

$$Chl_a = 12.25 * (A663) - 2.79 * (A647) \quad (3)$$

$$Chl_b = 21.50 * (A647) - 5.10 * (A663) \quad (4)$$

$$Total\ Chlorophyll = (Chl_a) + (Chl_b) \quad (5)$$

$$Carotenoids = 1000 * (A470) - 1.82 * Chl_a - 95.15 * Chl_b/225 \quad (6)$$

2.3. Enzyme activities

After 0.6 g of bamboo leaf samples were powdered with liquid nitrogen, pH 7.6 phosphate buffer was added to 4 mg of powders in separate tubes, followed by centrifugation at 3500–4500 × *g* at 6 °C for 22 min. The assessment of antioxidant enzyme activities was conducted using the final supernatant.

The Senthilkumar (Senthilkumar et al., 2021) methodology was used to photoreduce nitro blue tetrazolium (NBT) to measure the superoxide dismutase (SOD) activity. The OD was determined at 560 nm. Peroxidase activity (EC.1.11.1.6) was quantified following the modified protocol outlined by Zhang et al. (2012). To find the final POD, the absorbance at 436 nm was recorded using a molar absorption coefficient of 26.6 mM^{-1} cm^{-1}. The Catalase (CAT-1.11.1.7) activity (mg /g protein) was determined using the Aebi technique, Aebi, (1984), with measuring the rate of primary H_2O_2 degradation at 240 nm wavelength. Using Berner's approach (Berner et al., 2006), the activity of phenylalanine ammonia-lyase (PAL) was measured, and the ultimate PAL activity was quantified as U mg^{-1} of protein.

2.4. Antioxidant metabolism

The flavonoid concentration was determined per the Chang protocol at 510 nm (Chang et al., 2002) by using a standard curve. This value is expressed as mg rutin equivalents (RE)/g of plant material. The Kayden et al. (1973) method was employed to quantify tocopherol concentration. This entailed measuring the absorbance at 534 nm, comparing it to the tocopherol acetate standard calibration curve, and expressing the result as mg of tocopherol acetate equivalent per g of plant material. The concentration of total soluble phenol was also assessed following the method outlined by Imeh and Khokhar (2002). The absorbance at 650 nm was used to calculate the final concentration of total phenols, which was shown in mg of gallic acid g^{-1} F.W.

2.5. Spermidine, putrescine and proline measurements

The levels of spermidine (Spd) and putrescine (Put) was measured according to the H_2O_2 levels, which are generated as a result of polyamine oxidation (Zhao et al., 2004). The proline levels were determined based on the protocol by Bates et al., (1973).

2.6. Determination of shoot and root dry weight and shoot length

Clean bamboo shoots and roots were put in a vacuum-drying oven (DZF-6090) (Xiamen Tob New Energy Technology, Xiamen, China) and dried for 30 min. at 120° C to eliminate any remaining water. The oven were then set for 48 h at 78 °C to achieve consistent weight. Four replicates (including roots and shoots) were used to dry weight measurements. Following the end of the experiment, the shoot lengths of all four treatments were also measured.

2.7. Bioaccumulation and translocation factors and tolerance index

The method proposed by Souri and Karimi (2017) was employed for the computation of the Bioaccumulation Factor (BAF), Tolerance Index (TI), and Translocation Factor (TF). The BAF, TI, and TF calculations were performed using the following formula:

BAF = Hg level in the root or shoot of bamboo / Hg content in the medium (7)

TI of shoot = shoot dry weight / dry weight of the control (8)

TI of Root = root dry weight / dry weight of the control (9)

TF= Hg content in the bamboo leaves and stems / Hg content in the roots (10)

2.8. Statistics

This study was performed with a CRD in four replications. The Analysis of Variance (ANOVA) was conducted using the R software, employing a 2-way factorial design. Tukey's test was used at a probability level of $p < 0.05$ to analyze the mean difference among groups.

3. Results

3.1. Fe NPs and Fe conventional sources improve nutrient availability

The data analysis indicated a statistically significant difference ($p < 0.001$) in plant nutrient availability among different treatments in bamboo species. This demonstrated that the proportion of plant nutrients, such as calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), and nitrogen (N), increased with the addition of Fe NPs and other Fe sources. Adding Fe NPs, Fe S, and Fe C significantly increased plant nutrient availability compared to the control treatments. The combination of 60 mg/L Fe NPs alone and in combination with (Fe S + Fe C) increased plant nutrients in bamboo species under 80 mg/L Hg. The application of Fe NPs, Fe S, and Fe C resulted in a 76%, and 65% in nitrogen availability; 36%, and 32% increase in Mg availability; a 92%, and 75% in phosphorous availability, 61%, and 45% in potassium availability, 225%, and 185% in calcium availability, respectively, in comparison with the control treatments (Fig. 1). Under Hg toxicity, however, the results showed that combining Fe NPs + Fe S and Fe NPs + Fe C significantly enhanced nutrient availability. In contrast, bamboo treatments with 80 mg/L Hg exhibited the least availability, resulting in reductions of 32%, 34%, 34%, 21%, and 32% in N, Mg, P, K, and Ca, respectively. This suggests that the enhancement of the availability of plant nutrients in the presence of Hg toxicity may be beneficial when Fe NPs and Fe from conventional sources are added (Fig. 1).

3.2. Fe NPs and Fe conventional sources reduce Hg accumulation in bamboo organs

A significant difference occurred in Hg concentration in the shoot, root, and stems with addition of Fe NPs and conventional Fe sources ($p < 0.001$). Fe NP treatments alone, with (Fe S + Fe C) and (Fe S) showed the most significant drops in Hg concentration (54%, 49%, and 44% decreases in Hg concentration in the leaves, 57%, 48%, and 38% drops in the stems, and 48%, 42%, and 35% drops in the roots compared to control treatments). On the other hand, results revealed that introducing Fe NPs + Fe C, Fe S, Fe S + Fe C, and Fe C into plant organs (leaves, stem, and root) also resulted in a substantial reduction in Hg concentrations with 33%, 24%, 19%, and 11% reduction in leaves, 19%, 19%, 30%, and 8% reduction in stems, 29%, 22%, 18%, and 10% reduction in roots, respectively (Hg) (Fig. 2).

3.3. Fe NPs and Fe conventional sources improve cell membrane stability and relative water

The data indicated that the addition of Fe NPs, Fe S, and Fe C in combinations and single forms resulted in a significant difference between the different treatments in MSI and LRWC compared to the control treatments. ($p<0.001$). The highest boost in MSI and LRWC indexes was observed when Fe NPs were added either alone or with other Fe sources, which occurred in the presence of Hg. In contrast with control, the treatments including Fe NPs and the combination of Fe NPs + Fe S + Fe C; Fe NPs + Fe S; and Fe NPs + Fe C demonstrated the biggest rise in MSI and LRWC, with increases in MSI content by 46%, 41%, 34%, and 24% and increases in LRWC by 71%, 59%, 48%, and 38% compared to controls. Nevertheless, the data indicated that applying 60 mg/L Fe S and 60 mg/L Fe C led to a significant rise in MSI and LRWC levels. In particular, there was a 19%, and 10% increase in the MSI content and a 30%, and 10% increase in LRWC compared to their control treatments (80 mg/L Hg) (Fig. 3).

3.4. Fe NPs and Fe conventional sources improve photosynthetic pigments

A significant difference ($p<0.001$) in photosynthetic pigment levels across different treatments was detected. Fe NPs, Fe S, and Fe C significantly enhanced the photosynthetic pigments in bamboo species with and without Hg toxicity (Table 1). The results showed that while Fe NP treatment had the most highest impact on bamboo species in normal conditions (without Hg toxicity), 31%, 47%, 39%, and 46% decreases in chlorophyll a, chlorophyll *b*, total chlorophyll, and carotenoids was observed, respectively. The most significant increases in chlorophyll pigments under 80 mg/L Hg were associated with applying Fe NPs alone and in combination with Fe S + Fe C, which led to 110% and 96% rise in chlorophyll a, 41% and 51% rise in chlorophyll *b*, 74%, and 62% rise in total chlorophyll, and 148%, and 169% rise in carotenoids, respectively compared to the control treatments (Table 1).

3.5. Fe NPs and conventional Fe sources improve antioxidant enzyme activities

Adding Fe and other Fe sources significantly enhanced the

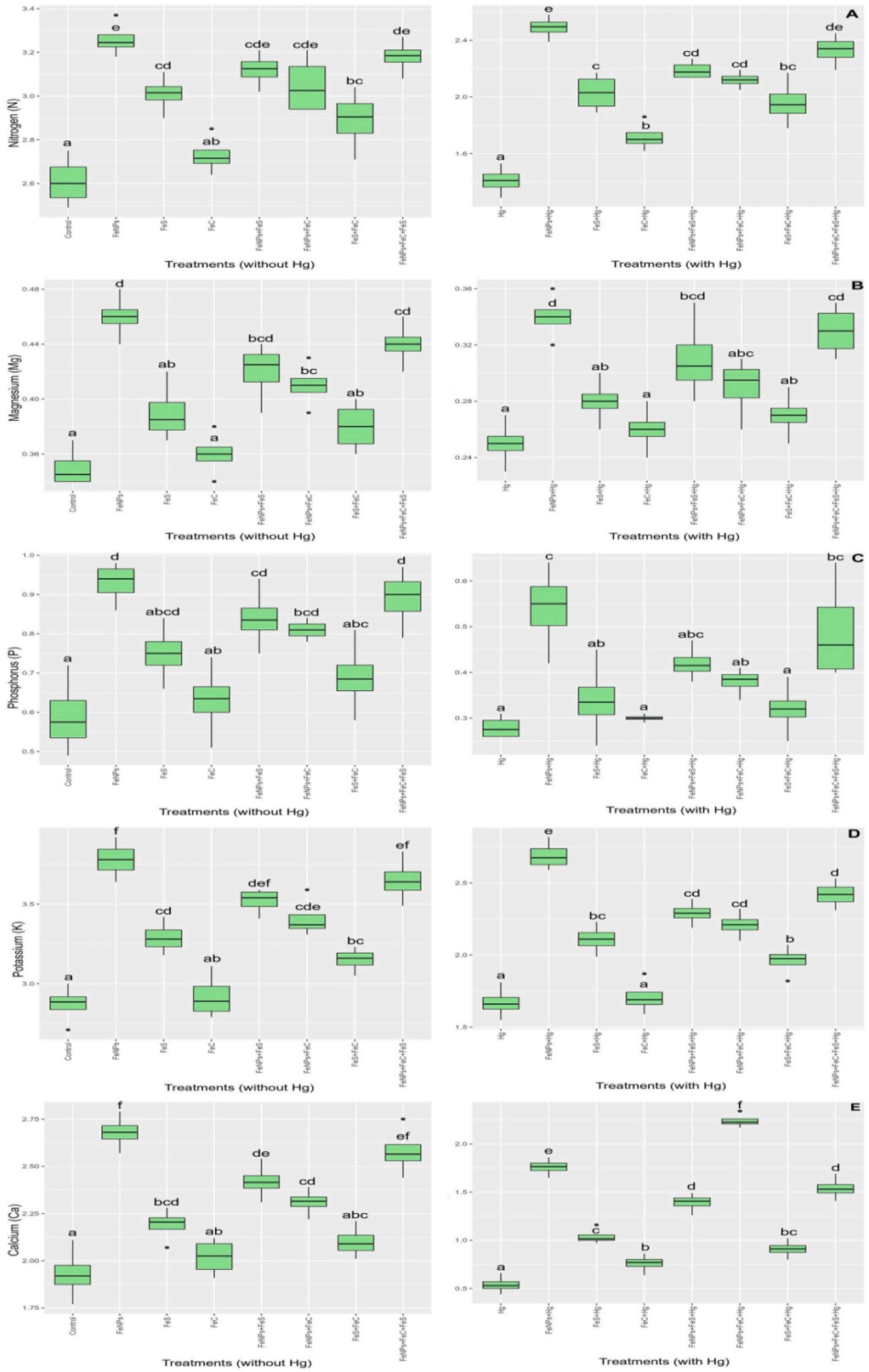

Fig. 1. The impact of 60 mg/L Fe NPs, Fe S, and Fe C individually and in combination with and without 80 mg/L Hg on plant nutrient availability: nitrogen **(A)**, magnesium **(B)**, phosphorous **(C)**, potassium **(D)**, and calcium **(E)**, in bamboo species (*Pleioblastus pygmaeus)*. The tretments are including, control (no Hg and no Fe); 60 mg L^{-1} FeNPs; 60 mg L^{-1} FeS; 60 mg L^{-1} FeC; 60 mg L^{-1} FeNPs+FeS; 60 mg L^{-1} FeNPs+FeC; 60 mg L^{-1} FeS+FeC; 60 mg L^{-1} FeNPs+FeS+FeC; 80 mg L^{-1} Hg; 60 mg L^{-1} FeNPs+80 mg L^{-1} Hg; 60 mg L^{-1} FeS+80 mg L^{-1} Hg; 60 mg L^{-1} FeC+80 mg L^{-1} Hg; 60 mg L^{-1} FeNPs+FeS+80 mg L^{-1} Hg; 60 mg L^{-1} FeNPs+FeC+80 mg L^{-1} Hg; 60 mg L^{-1} FeS+FeC+80 mg L^{-1} Hg; and 60 mg L^{-1} FeNPs+FeS+FeC+80 mg L^{-1} Hg. Boxes depict the lower and upper quartiles (25–75%). The whiskers represent the min–max values. Lines in the boxes indicate median values. a, b, c. etc. letters demonstrate significant differences between FeNPs, FeS, and FeC in single and combined form under 0 and 80 mg/L Hg. Tukey, (n=4) ($p < 0.05$).

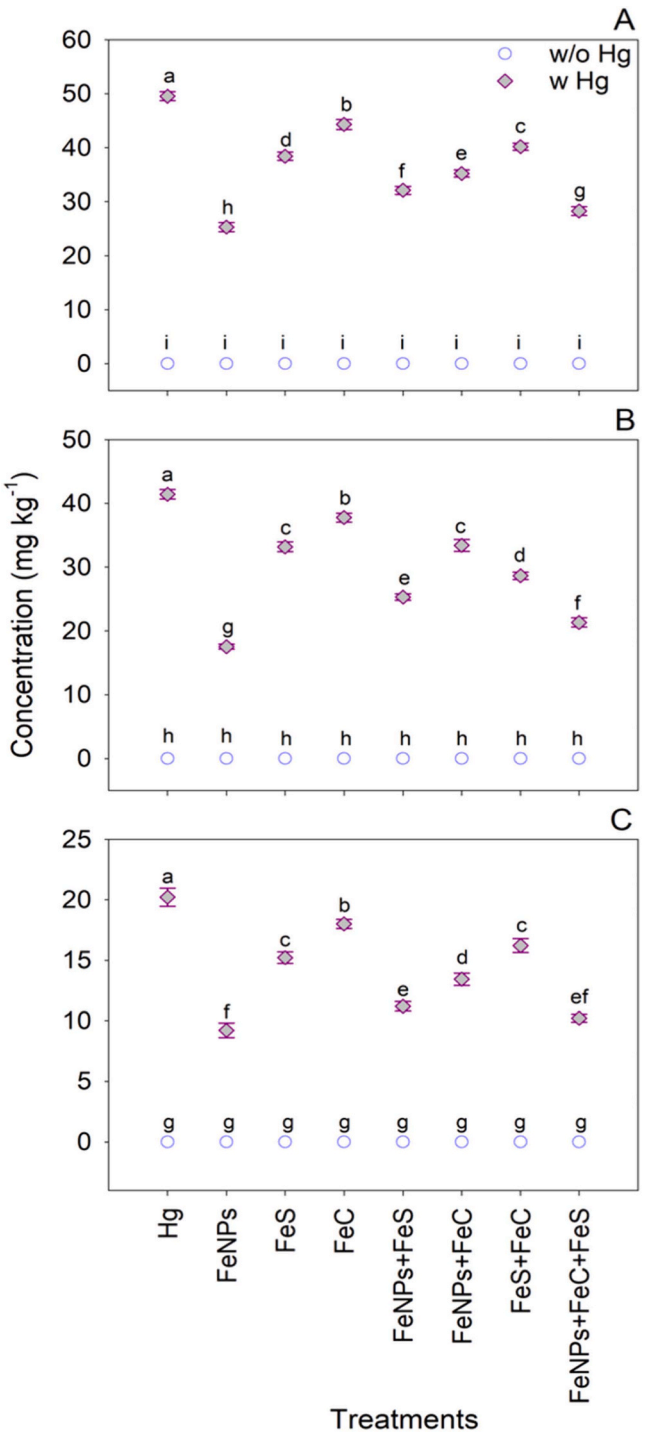

Fig. 2. Mercury levels in bamboo organs: (**A**) leaves, (**B**) stem, and (**C**) root. The data display mean ± standard error (n=4). The treatments comprise single and combined 60 mg/L Fe NPs, Fe S, and Fe C with 80 mg/L Hg. a, b, c. etc. letters demonstrate significant differences between FeNPs, FeS, and FeC in single and combined form under 80 mg/L Hg Tukey, $p < 0.05$).

antioxidant capacity of bamboo plant species with and without Hg toxicity. This increase was shown to be statistically significant ($p < 0.001$). The addition of Fe NPs and other Fe sources (Fe S and Fe C) led to a significant increase in antioxidant activity indexes, either alone or in combination. The most pronounced increase in antioxidant activity was observed in the Fe NP treatments, which exhibited an average increase of 31% in SOD activity, 30% in POD activity, 37% in CAT activity, and 50% in PAL activity. The individual form of Fe NPs and the combination of three different iron sources ((Fe NPs + Fe S + Fe C), (Fe NPs + Fe S), and (Fe NPs + Fe C)) demonstrated the most pronounced effects on enhancing antioxidant activity in bamboo plants under Hg toxicity. These combinations resulted in a 44%, 35%, 28%, and 25% increase in SOD activity, a 40%, 31%, 25%, and 21% increase in POD activity, a 29%, 26%, 23%, and 20% increase in CAT activity, and a 168%, 148%, 108%, and 82% increase in PAL activity compared to the control treatments, respectively. However, individual forms of Fe S and Fe C resulted in enhanced antioxidant activity in plants exposed to 80 mg/l Hg toxicity. Specifically, there was an 18% increase in SOD activity, a 15% increase in POD activity, a 15% increase in CAT activity, and a 50% increase in PAL activity compared to the controlsfor Fe S. Similarly, Fe C exhibited a 7% increase in SOD activity, a 5% increase in POD activity, a 4% increase in CAT activity, and an 18% increase in PAL activity compared to the control treatments (Fig. 4).

3.6. Fe NPs and conventional Fe sources improve antioxidant metabolism

Antioxidant metabolism indices of bamboo plants with and without Hg toxicity showed a significant difference ($p < 0.001$) between treatments after the addition of Fe NPs, Fe S, and Fe C. Nevertheless, findings did not exhibit any substantial disparity in the flavonoids in the presence of Hg toxic exposure. However, there was a statistically significant difference in the flavonol concentrations without Hg toxicity (Fig. 5). The flavonoid index exhibited the greatest increase in response to the Fe NPs treatments, with a 38% increase relative to the control treatment. Conversely, our data confirmed that other antioxidant metabolism indexes, including tocopherol, and total phenolics, were significantly enhanced by applying Fe NPs and other Fe sources. Compared to control treatments, adding Fe NPs alone and combined with Fe S + Fe C showed the biggest rise in antioxidant metabolism under Hg toxicity. Fe NPs alone increased tocopherol levels by 49% and total phenolics by 28% compared with controls. On the other hand, co-application of Fe NPs + Fe S, Fe NPs + Fe C, and Fe S + Fe C improved antioxidant activity in plants subjected to Hg toxicity. There was a 16% increase in tocopherols and a 10% increase in total phenolics when Fe NPs + Fe S were applied. Similarly, the application of Fe NPs + Fe C resulted in a 26% increase in tocopherols and an 11% increase in total phenolics. Lastly, the combination of Fe S + Fe C contributed to a 16% increase in tocopherols and a 9% increase in total phenolics compared to the control treatments. In contrast, individual forms of Fe S and Fe C led to an elevation in antioxidant metabolism by a 19%, and 6% increase in tocopherols and a 12%, and 5% increase in total phenolics, corresponding to the control treatments (Fig. 5).

3.7. Fe NPs and conventional Fe sources improve polyamines and proline

The addition of Fe NP_S, Fe S, and Fe C to bamboo species exposed to Hg toxicity resulted in a significantly higher concentration of spermidine and putrescine across treatments, accompanied by an accumulation of proline ($p < 0.001$). This was also observed in the bamboo treatment without Hg toxicity, which demonstrated a significant difference between treatments in spermidine, putrescine, and proline ($p < 0.001$). Treatments involving the addition of Fe NPs alone or in combination with Fe S + Fe C resulted in the highest rises in these indices for bamboo plants without Hg exposure or subjected to Hg levels below 80 mg/l. The concentrations of spermidine and putrescine were found to have increased by 220% and 184%, respectively, in the presence of Hg toxicity, while the accumulation of proline exhibited an increase of 52%, and 44% in comparison to the control group. On the contrary, the combined Fe NP_S + Fe S, Fe NP_S + Fe C, and Fe S + Fe C exhibited a substantial growth in the accumulation of spermidine, putrescine, and proline in plants exposed to Hg. Specifically, there was a significant increase in the accumulation of spermidine (162%), putrescine (124%), and proline (37%) when Fe NP_S + Fe S was present. Similarly, Fe NP_S + Fe C resulted in a significant increase in the accumulation of putrescine

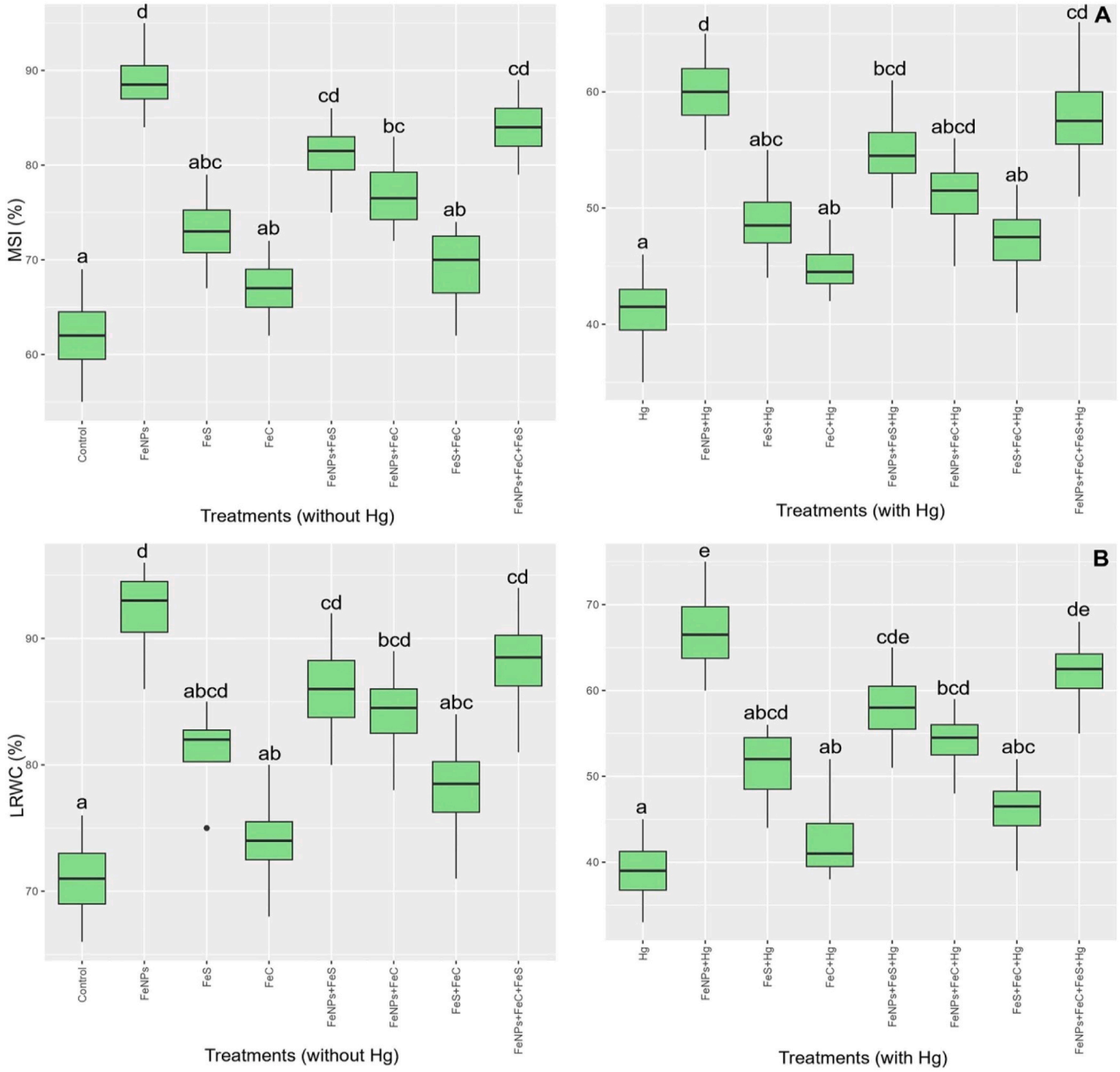

Fig. 3. The impact of 60 mg/L Fe NPs, Fe S, and Fe C individually and in combination with and without 80 mg/L Hg on MSI% **(A)** and LRWC **(B)** in bamboo species (*Pleioblastus pygmaeus)*. The tretments are including, control (no Hg and no Fe); 60 mg L^{-1} FeNPs; 60 mg L^{-1} FeS; 60 mg L^{-1} FeC; 60 mg L^{-1} FeNPs+FeS; 60 mg L^{-1} FeNPs+FeC; 60 mg L^{-1} FeS+FeC; 60 mg L^{-1} FeNPs+FeS+FeC; 80 mg L^{-1} Hg; 60 mg L^{-1} FeNPs+80 mg L^{-1} Hg; 60 mg L^{-1} FeS+80 mg L^{-1} Hg; 60 mg L^{-1} FeC+80 mg L^{-1} Hg; 60 mg L^{-1} FeNPs+FeS+80 mg L^{-1} Hg; 60 mg L^{-1} FeNPs+FeC+80 mg L^{-1} Hg; 60 mg L^{-1} FeS+FeC+80 mg L^{-1} Hg; and 60 mg L^{-1} FeNPs+FeS+FeC+80 mg L^{-1} Hg. Boxes depict the lower and upper quartiles (25–75%). The whiskers represent the min–max values. Lines in the boxes indicate median values. a, b, c. etc. letters demonstrate significant differences between FeNPs, FeS, and FeC in single and combined form under 0 and 80 mg/L Hg. Tukey, (n=4) ($p < 0.05$).

(122%), spermidine (102%), and proline (30%). Furthermore, the combination of Fe S + Fe C resulted in an 18% increase in proline and 46% and 80% increases in putrescine and spermidine accumulation, respectively, in comparison to the control treatments (Suppl Fig. 1).

3.8. Fe NPs and Fe conventional sources improve shoot and root dry weight and shoot length

The shoot and root dry weight and shoot length, were used to evaluate plant biomass and assess plant development. A significant difference ($p < 0.001$) was observed in plant biomass and plant growth indices between different treatments, irrespective of the presence or absence of Hg toxicity. The shoot dry weight data did not reveal significant differences between treatments under normal conditions (non-Hg toxicity), however, root dry weight and shoot length significantly increased with the addition of Fe NPs and conventional Fe sources (Fe S, and Fe C) (Suppl Fig. 2). The data revealed that the greatest increase in root dry weight and shoot length was observed in plants treated with Fe NPs, which exhibited 20% and 38% increases, respectively. These findings suggest that the introduction of Fe NPs and other forms of iron, whether as combined treatments or individual substances, to Hg - exposed plants can enhance shoot and root dry weight, as well as shoot

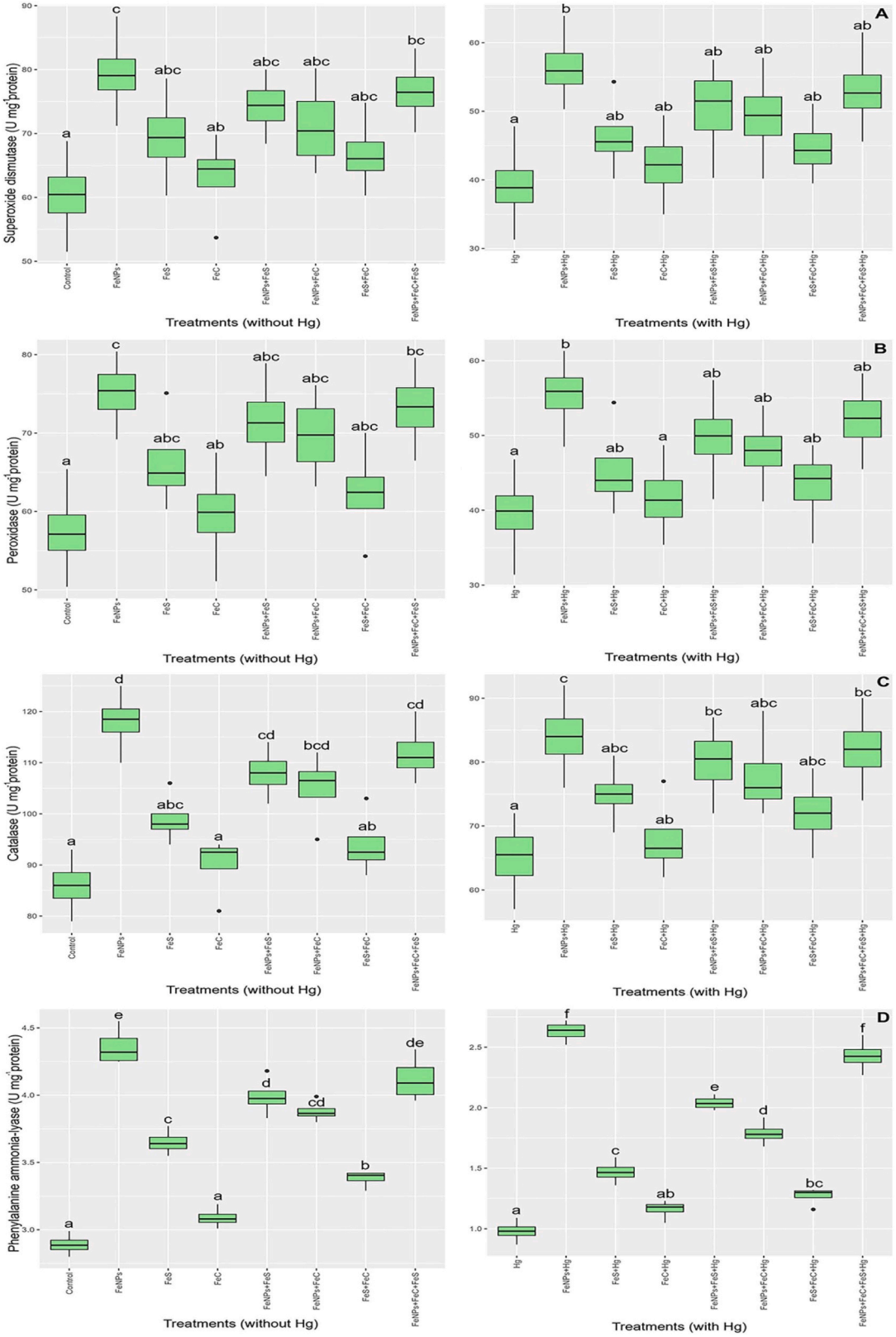

Fig. 4. The impact of 60 mg/L Fe NPs, Fe S, and Fe C individually and in combination with and without 80 mg/L Hg on antioxidant enzymes activity; SOD **(A)**, POD **(B)**, CAT **(C)**, and PAL **(D)** in bamboo species (*Pleioblastus pygmaeus)*. The tretments are including, control (no Hg and no Fe); 60 mg L^{-1} FeNPs; 60 mg L^{-1} FeS; 60 mg L^{-1} FeC; 60 mg L^{-1} FeNPs+FeS; 60 mg L^{-1} FeNPs+FeC; 60 mg L^{-1} FeS+FeC; 60 mg L^{-1} FeNPs+FeS+FeC; 80 mg L^{-1} Hg; 60 mg L^{-1} FeNPs+80 mg L^{-1} Hg; 60 mg L^{-1} FeS+80 mg L^{-1} Hg; 60 mg L^{-1} FeC+80 mg L^{-1} Hg; 60 mg L^{-1} FeNPs+FeS+80 mg L^{-1} Hg; 60 mg L^{-1} FeNPs+FeC+80 mg L^{-1} Hg; 60 mg L^{-1} FeS-+FeC+80 mg L^{-1} Hg; and 60 mg L^{-1} FeNPs+FeS+FeC+80 mg L^{-1} Hg. Boxes depict the lower and upper quartiles (25–75%). The whiskers represent the min–max values. Lines in the boxes indicate median values. a, b, c. etc. letters demonstrate significant differences between FeNPs, FeS, and FeC in single and combined form under 0 and 80 mg/L Hg. Tukey, (n=4) ($p < 0.05$).

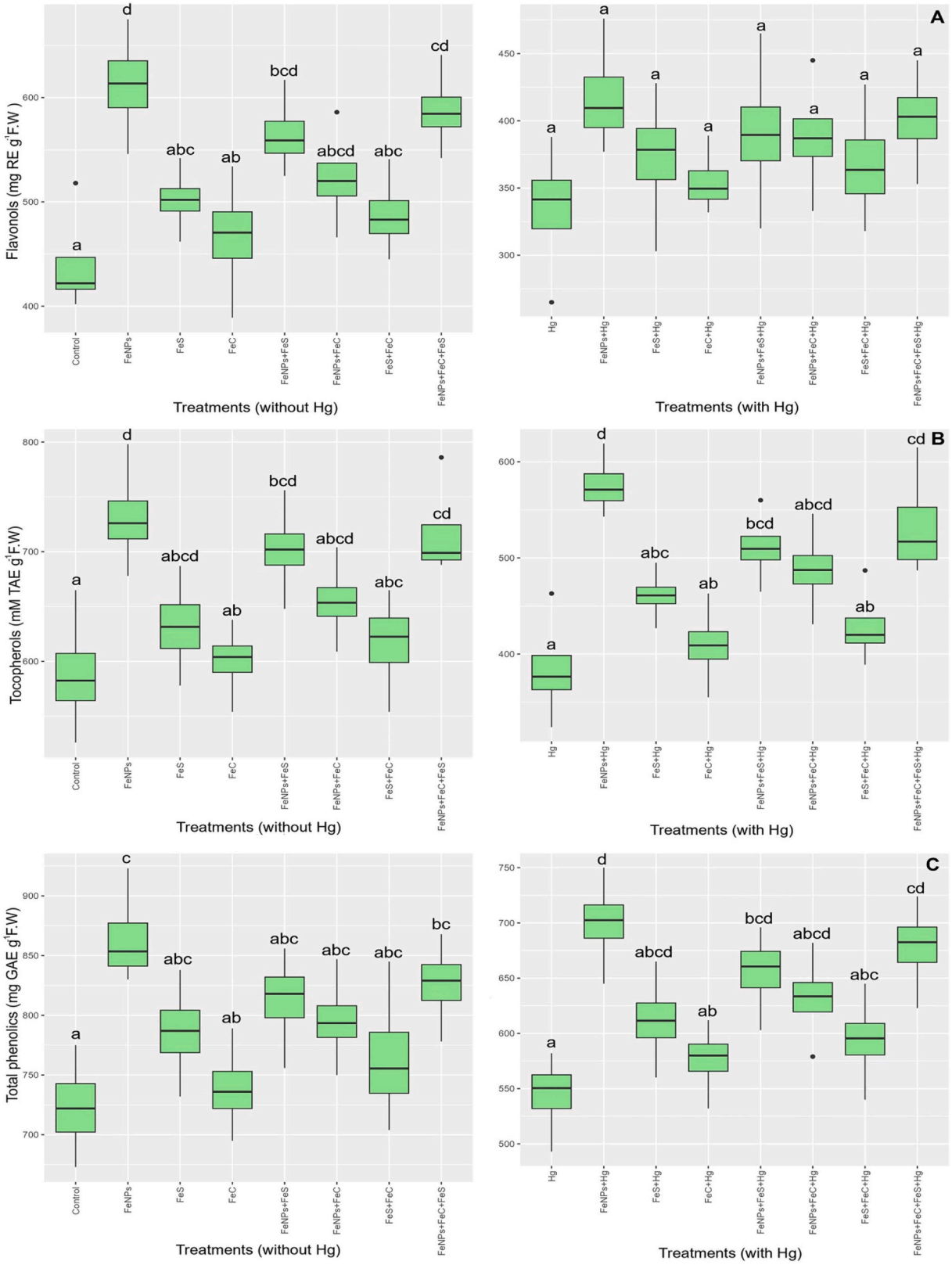

Fig. 5. The impact of 60 mg/L Fe NPs, Fe S, and Fe C individually and in combination with and without 80 mg/L Hg on antioxidant metabolism indexes; Flavonols **(A)**, Tocopherols **(B)**, and total phenolics **(C)** in bamboo species (*Pleioblastus pygmaeus)*. The tretments are including, control (no Hg and no Fe); 60 mg L^{-1} FeNPs; 60 mg L^{-1} FeS; 60 mg L^{-1} FeC; 60 mg L^{-1} FeNPs+FeS; 60 mg L^{-1} FeNPs+FeC; 60 mg L^{-1} FeS+FeC; 60 mg L^{-1} FeNPs+FeS+FeC; 80 mg L^{-1} Hg; 60 mg L^{-1} FeNPs+80 mg L^{-1} Hg; 60 mg L^{-1} FeS+80 mg L^{-1} Hg; 60 mg L^{-1} FeC+80 mg L^{-1} Hg; 60 mg L^{-1} FeNPs+FeS+80 mg L^{-1} Hg; 60 mg L^{-1} FeNPs+FeC+80 mg L^{-1} Hg; 60 mg L^{-1} FeS+FeC+80 mg L^{-1} Hg; and 60 mg L^{-1} FeNPs+FeS+FeC+80 mg L^{-1} Hg. Boxes depict the lower and upper quartiles (25–75%). The whiskers represent the min–max values. Lines in the boxes indicate median values. a, b, c. etc. letters demonstrate significant differences between FeNPs, FeS, and FeC in single and combined form under 0 and 80 mg/L Hg. Tukey, (n=4) ($p< 0.05$).

length. However, the treatments that included Fe NPs, either alone or in combination with Fe S + Fe C, Fe NPs + Fe C, and Fe NPs + Fe S, had the highest increase in biomass and plant growth. These treatments resulted in a 42%, 33%, 28%, and 20% increase in shoot dry weight, respectively, compared to the control. Fe NPs in both individual and combined forms with Fe S and Fe C, as well as the combination of Fe NPs with Fe C and Fe S, exhibited the most substantial enhancement in biomass and plant growth. More precisely, the dry weight of the root increased by 40%, 36%, 31%, and 25%. In addition, the shoot length had respective increases of 42%, 37%, 30%, and 22%. Conversely, as predicted, the treatments containing 80 mg/l of Hg exhibited the lowest plant biomass and growth levels. There was a 36% decrease in bamboo shoot dry weight, a 31% reduction in root dry weight, and a 31% decrease in shoot length compared to controls (Suppl Fig. 2) (Suppl Table 2).

3.9. Fe NPs and conventional Fe sources reduce bioaccumulation and translocation factors and increase the tolerance index

The results indicate that adding Fe NPs and other Fe sources significantly affected the reduction of Hg accumulation in many parts of the bamboo plant, including the leaves, stem, and root. Furthermore, there was a statistically significant variation seen among the different treatments in terms of bioaccumulation factor (BAF) ($p<0.001$). Fe NPs alone and combined with iron carbide and iron sulfide (Fe NPs + Fe C + Fe S) resulted in the most significant decrease in Hg accumulation. Precisely, Hg accumulation in leaves decreased by 54%, in stems by 49%, and in roots by 48% compared to the control groups. In contrast, the addition of Fe NPs and conventional Fe sources was observed to significantly decrease Hg translocation from the roots to the stem. This observation highlights the potential of Fe NPs and other conventional Fe sources in mitigating Hg translocation to plant aerial parts. Nevertheless, the amount of translocated element was negligible for the leaves. The treatment that caused the most substantial decrease in TF of the stem was observed when Fe NPs were employed alone, as well as in combination with Fe C and Fe C + Fe S, resulting in decreases of 17%, 14%, and 10% respectively, compared to the controls.

Furthermore, TI calculation was used to examine the contribution of Fe NPs and other Fe sources in enhancing bamboo Hg tolerance. The single and combination forms of Fe NPs, Fe S, and Fe C exhibited a statistically significant difference across treatments ($p < 0.001$) under Hg toxicity. However, the treatments under normal conditions did not show any significant difference in the TI of shoots and roots. However, the groups receiving treatments with Fe NPs, Fe NPs + Fe S + Fe C, and Fe NPs + Fe S showed the most significant improvements in plant tolerance. The treatments led to an increase of 42%, 34%, and 29% in shoot TI, and a rise of 41%, 37%, and 32% in root TI, compared to the control treatments (Table 2).

4. Discussion

We conducted a study in a controlled greenhouse to investigate the effects of different types of iron, including Fe NPs, Fe S, and Fe C, on the biomass, growth physiology, and cell integrity of Hg exposed bamboo plants. Our aim was to understand how these iron types can potentially enhance the plants' ability to tolerate Hg. According to prior research, toxic Hg exposure can reduce the availability of critical plant nutrients and interfere with nutrient absorption (Alengebawy et al., 2021; Vasilachi et al., 2023; Mashabela et al., 2023). According to Boening (2000), the presence of Hg, whether in inorganic or organic form, was associated with the depletion of Mg, K, and Mn, as well as the contribution of Fe accumulation. Conversely, by lowering the pH of HM-affected soil, Fe NPs might increase the availability of vital nutrients like zinc and phosphorus (Yoon et al., 2019). According to a recent study conducted by Zia-ur-Rehman et al. (2023b), Fe's presence enhances the absorption of vital nutrients through root interception, particularly under cellular stress. Our findings proved that, adding Fe NPs and other easily accessible sources of Fe significantly increases plant nutrient availability in Hg exposed bamboo species. The presence of Fe NPs can facilitate the dissolution of insoluble compounds, such as Ca complexes, in the soil. This process involves the interaction of Fe NPs with other elements, like phosphorous, to prevent structural soil damage. Additionally, Fe NPs can aid in stabilizing soil structure and replacing adsorbed Na ions (De Souza et al., 2019). Our results indicate that the presence of Fe NPs and other sources of Fe decreases the availability of Hg in plants and soil. This reduction is attributed to the NP's ability to absorb ions, which was previously described in one of our works. Emamverdian et al. (2021a) showed that NPs, specifically Fe NPs, possess a higher surface-to-weight ratio and a larger surface area. This unique characteristic enables them to interact with metal ions and facilitate ion absorption effectively. Consequently, the presence of Fe NPs results in a reduction of Hg accumulation and Hg translocation inside plant organs. Here, incorporating Fe NPs significantly decreased the Hg concentrations in bamboo organs (root, stem, and leaves). Consequently, the translocation of Hg from the roots to the stems and leaves was effectively restricted. The increase in plant nutrient availability can be attributed to reduced plant Hg absorption Hg. Fe NPs can also absorb Hg ions in the root suface and reduce Hg translocation to the shoot and aerial plant parts, which can be one of the involved mechanisms in reducing Hg absorption.

Table 2
Changes in the translocation factor of leaves and stem, bioaccumulation factor of leaves, stem, and roots, and tolerance index of shoot and roots in bamboo plants (*Pleioblastus pygmaeus*) upon the addition of 60 mg L^{-1} Fe NPs, Fe C, and Fe S individually or in combination form under 80 mg L^{-1} Hg. The data display the mean ± standard error of four replicates. a, b, c. etc. letters demonestrate significant differences among Fe NPs, Fe S, and Fe C in single and combined form under 0 and 80 mg/L Hg (Tukey, $p < 0.001$).

Treatments	TF (leaves)	TF (stem)	BAF (leaves)	BAF (stem)	BAF(root)	TI(shoot)	TI(root)
control	0	0	0	0	0	1 ± 0.017^{aa}	0.99 ± 0.057^{a}
60 mg L^{-1} FeNPs	0	0	0	0	0	1.12 ± 0.07^{a}	1.2 ± 0.057^{a}
60 mg L^{-1} FeS	0	0	0	0	0	1.06 ± 0.07^{a}	1.08 ± 0.057^{a}
60 mg L^{-1} FeC	0	0	0	0	0	1.02 ± 0.07^{a}	1.02 ± 0.057^{a}
60 mg L^{-1} FeNPs+FeS	0	0	0	0	0	1.09 ± 0.07^{a}	1.14 ± 0.057^{a}
60 mg L^{-1} FeNPs+FeC	0	0	0	0	0	1.07 ± 0.007^{a}	1.09 ± 0.057^{a}
60 mg L^{-1} FeS+FeC	0	0	0	0	0	1.04 ± 0.07^{a}	1.05 ± 0.057^{a}
60 mg L^{-1} FeNPs+FeS+FeC	0	0	0	0	0	1.11 ± 0.07^{a}	1.16 ± 0.057^{a}
80 mg L^{-1} Hg	0.407 ± 0.01^{a}	0.836 ± 0.01^{cd}	0.25 ± 0.006^{f}	0.51 ± 0.008^{g}	0.61 ± 0.009^{f}	0.64 ± 0.05^{a}	0.69 ± 0.053^{a}
60 mg L^{-1} FeNPs+80 mg L^{-1} Hg	0.367 ± 0.01^{a}	0.696 ± 0.01^{a}	0.11 ± 0.006^{a}	0.21 ± 0.008^{a}	0.31 ± 0.009^{a}	0.90 ± 0.05^{b}	0.97 ± 0.05^{b}
60 mg L^{-1} FeS+80 mg L^{-1} Hg	0.395 ± 0.01^{a}	0.863 ± 0.01^{de}	0.19 ± 0.001^{cd}	0.414 ± 0.008^{e}	0.48 ± 0.009^{cd}	0.74 ± 0.05^{ab}	0.84 ± 0.053^{ab}
60 mg L^{-1} FeC+80 mg L^{-1} Hg	0.406 ± 0.01^{a}	0.851 ± 0.01^{d}	0.22 ± 0.006^{ef}	0.47 ± 0.008^{f}	0.55 ± 0.009^{e}	0.67 ± 0.05^{ab}	0.74 ± 0.053^{ab}
60 mg L^{-1} FeNPs+FeS +80 mg L^{-1} Hg	0.35 ± 0.01^{a}	0.790 ± 0.01^{bcd}	0.14 ± 0.006^{ab}	0.31 ± 0.008^{c}	0.40 ± 0.009^{b}	0.82 ± 0.05^{ab}	0.91 ± 0.05^{ab}
60 mg L^{-1} FeNPs+FeC +80 mg L^{-1} Hg	0.38 ± 0.01^{a}	0.948 ± 0.01^{e}	0.16 ± 0.006^{bc}	0.417 ± 0.008^{e}	0.44 ± 0.009^{bc}	0.77 ± 0.05^{ab}	0.87 ± 0.05^{ab}
60 mg L^{-1} FeS+FeC +80 mg L^{-1} Hg	0.402 ± 0.01^{a}	0.712 ± 0.01^{ab}	0.20 ± 0.006^{de}	0.35 ± 0.008^{d}	0.50 ± 0.009^{d}	0.71 ± 0.05^{ab}	0.78 ± 0.053^{ab}
60 mg L^{-1} FeNPs+FeS+FeC+80 mg L^{-1} Hg	0.36 ± 0.01^{a}	0.753 ± 0.01^{abc}	0.12 ± 0.006^{a}	0.26 ± 0.008^{n}	0.35 ± 0.009^{a}	0.85 ± 0.05^{ab}	0.94 ± 0.05^{b}

Hg leads to oxidative stress in plants by inducing reactive ROS compounds (H_2O_2), which, in turn, decreases the activity of H_2O_2 scavenging enzymes such as POX, and CAT (Sahu et al., 2012). The Hg was found to disrupt the regulation of enzymatic and non - enzymatic antioxidants in plants, including SOD, POD, CAT, GSH, APX, and GR. This interference affects the overall functioning of the antioxidant defence system in plants (Rodriguez et al., 2011). We also observed a reduction in the stimulation of antioxidant activity in bamboo following the reaction of Hg on the regulation of antioxidant activity in our previous experiments. On the other hand, studies by Sun et al. (2023) and Jalali et al. (2016) indicated that Fe NPs and other standard Fe types can enhance plants' antioxidant capacity, corroborating our data. Also, Fe NPs ameliorate As-induced oxidative stress by increasing the antioxidant defence capacity and the glyoxalase system (Bidi et al., 2021). Our data suggest that the antioxidant capacity, as measured by the levels of SOD, POD, CAT, and PAL, exhibited an increase in bamboo plants exposed to Hg toxicity when supplemented with Fe NPs, Fe S, and Fe C, both individually and in combination. A similar result was shown by Zia-ur-Rehman et al. (2023a) on wheat plants. The decrease in oxidative stress by incorporating Fe NPs and other Fe sources was associated with enhancing Fe absorption, reducing Hg accumulation, and boosting antioxidant activity. This ultimately helps scavenge ROS compounds and alleviate oxidative stress in bamboo plants.

In plants, phenolic content, such as phenolic acids and flavonoids having hydroxyl groups and aromatic rings are in the same direction as antioxidant enzyme activity. Therefore, their redox characteristics can drive plant antioxidant production (Ali-Arab et al., 2022). Oxidizing ROS compounds by phenolic metabolites is one of the mechanisms in modulating oxidative stress response in plants exposed to HMs (Emamverdian et al., 2022). Our current data showed that adding Fe NPs, Fe C, and Fe S in both single and combination forms had positive impact on increasing phenolic content, including total phenolics, flavonols, and tocopherols under Hg stress (induction of secondary metabolites in plants). However, the results obtained by flavonoid content were insignificant for treatments under Hg toxicity which was reported by another study (Jańczak-Pieniążek et al., 2022) on winter wheat plants. The accumulation of phenolic content by the Fe application was reported by several studies (Rezayian et al., 2023; Emamverdian et al., 2023). The presence of carboxyl and hydroxyl groups in phenolic compounds may be associated with the concentration of Fe. Furthermore, it was documented that phenols have the ability to reduce the level of superoxide produced by the Fenton reaction by facilitating the release of chelating Fe ions (Rezayian et al., 2023). The study conducted by Nourozi et al. (2019) observed an upregulation of genes related to the phenylpropanoid pathway in *Dracocephalum kotschyi*. This was achieved by treating the plants with Fe NPs, resulting in increased phenolic and flavonoid contents. Additionally, the treatment with Fe NPs improved the stability of plant membranes when exposed to HM stress. The excessive ROS formation induced by HMs significantly diminishes the integrity of the plant cell membrane. This occurs through the interaction between ROS and the cellular membranes, resulting in an elevation of electrolyte leakage and detrimental effects on the chlorophyll's thylakoid membrane (Aazami et al., 2023; Sachdev et al., 2021). According to Ma et al. (2023), the impairment of the thylakoid membrane leads to a decrease in chlorophyll levels and ultimately hampers photosynthesis in the target plant. In this study, the augmentation of both antioxidant and non - antioxidant activity induced by Fe in plants may effectively eliminate ROS inside the plants subjected to Hg exposure. This mechanism serves to protect the integrity of the cell membranes and enhances cell stability under stress. Our data shows that the stability of bamboo species exposed to Hg was protected by Fe NPs, Fe S, and Fe C. This finding is consistent with the study by Mozafari et al. (2018b) on strawberry plants under salt stress, as well as the work performed by Zia-ur-Rehman et al. (2023a) on wheat plants under HM stress. Mercury can potentially restrict chlorophyll synthesis, decrease the rate of transpiration, and disrupt photosynthesis (Mei et al., 2021). The studies have indicated that Hg significantly diminishes the water intake as well (Boening, 2000). In contrast, Fe is a prominent element found in enzymes that play a crucial role in the photosynthesis, hence enhancing the plant's capacity to harness and absorb light energy effectively (Zia-ur--Rehman et al., 2023a). Iron, as a vital micronutrient for plants, also plays a crucial function in facilitating photosynthetic processes by contributing to the equilibrium of photosystem I and II compartments (Rai et al., 2022). Several studies have provided data indicating that Fe NPs possess the ability to chelate ROS, hence exhibiting enhanced antioxidant capacity. This, in turn, leads to increased efficiency of photosynthesis in plants (Babashpour-Asl et al., 2022; Sheykhbaglou et al., 2010). Our study demonstrated that Hg presence leads to a decrease in photosynthetic pigments. However, we observed that introducing Fe in the form of three types of nanoparticles, (Fe NPs, Fe C, and Fe S)increased chlorophyll **a**, chlorophyll ***b***, total chlorophyll, and carotenoids in bamboo plants exposed to Hg toxicity. The reason behind this can be related to the role of Fe as a source of iron in metabolic reactions. Because, once absorbed by the roots and carried to the leaves, Fe leads to an increase in chlorophyll content in wheat (Al-Amri et al., 2020). Zia-ur-Rehman et al. (2023a) documented similar findings on the impact of several Fe sources, including Fe NPs, Fe SO_4, and Fe - EDTA, on wheat plants subjected to salt stress.

Proline, as one of the aliphatic amino acids, protects plant species from various forms of biotic and abiotic stressors. Consequently, proline facilitates the rapid recovery of plants from stress (Hayat et al., 2012). The regulation of plant development under stress can be altered by the proline synthesis (Torabian et al., 2016). The process is crucial for ROS scavenging, maintaining membrane integrity, and mitigating osmotic stress (Bandurska, 2001). Our data suggest that the presence of Fe NPs and other conventional Fe sources may lead to an increase in proline levels in bamboo plants exposed to Hg toxicity. This phenomenon might be explained by the involvement of NPs in regulating the expression of genes related to proline biosynthesis. Increasing the synthesis of proline can aid in maintaining the level of water in the leaves (Sardar et al., 2022), which is associated with the activity of genes responsible for regulating water content in plants (Koleva et al., 2022). We previously reported that utilizing ZnO - NPs with the inclusion of Fe NPs reduced Cu and Cd toxicity while boosting proline accumulation (Emamverdian et al., 2021a; ; Bidi et al., 2021; Koleva et al., 2022; Seshagiri et al., 2024).

The RWC in leaves serves as a significant indicator for assessing the impact of HM stress levels on plants, as highlighted by Ahmad et al. (2018). Therefore, evaluating the plant's water status under stress is one of the primary physiological stress response markers (Sarker and Oba, 2018). Based on our data, Fe NPs and other Fe sources affected water content in bamboo plant cells under Hg stress. This impact was mainly attributed to the increase in RWC percentage and the optimization of metabolic processes. Consequently, these effects contributed to the promotion of plant development. Fe NPs and other Fe forms offer a convenient approach to mitigate the toxicity of HMs. Here, this was achieved by impeding the excessive reduction of Fe inside the leaves, hence keeping the structural integrity of the bamboo leaves. Consequently, preservation of leaf structure contributes to the effective management of plant water content (Shirani Bidabadi et al., 2023). There was a positive relationship between the Fe addition and increased RWC rates in *Glycine max* leaves according to Dola et al. (2022) with the introduction of nano - iron.

Polyamines regulate plant growth and reproduction through cellular functions under conditions of HM stress (Malik et al., 2022). Spormann et al. (2021) documented that gene expressions associated with detoxification via vacuole compartmentalization are attributable to polyamines. Putrescine (Put) and spermidine (Spd) are prominent polyamines identified as diamines within plant cells. Malik et al. (2022) reported that polyamines actively engage in membrane integrity and stress tolerance. Here, our conclusions indicate that introducing Fe NPs, Fe C, and Fe S has a remarkable positive impact on the levels of Spd and

Put in plants subjected to Hg toxicity. Koleva et al. (2022) indicated that the concentration of Put and Spd increased under Cd stress, when iron oxide NPs were added. This aligns with our results obtained in bamboo species under Hg stress. The observed elevation emphasized the importance of Fe NPs and other Fe sources in moderating the harmful effects of Hg in bamboo plants.

On the other hand, ROS production in plants results in the breakdown of plant defence systems, impairing photosynthesis, and constraining plant growth (Rizwan et al., 2019). According to Moussa et al. (2010), incorporating Fe NPs and other conventional types of Fe can enhance the nutritional concentration of essential phytochemicals, which are known to facilitate plant growth and development. Rout and Sahoo (2015) documented that the presence of Fe in plants promotes cell division, resulting in the expansion of cells. Our data demonstrated that introducing Fe NPs, Fe C, and Fe S significantly enhanced plant biomass and growth parameters, such as shoot dry weight, root dry weight, and shoot length, in the presence of Hg toxicity. The promotion might be related to the role of Fe as an essential nutrient for plant maintenance in the reduction of Hg toxicity which can help sustain plant growth and regulation. It also might be attributed to the enhancement of bamboo photosynthesis under Hg toxicity since Fe is essential for chloroplast structure and function. Previous data documented the involvement of Fe NPs and other Fe forms in plant growth and development as well (Rizwan et al., 2019; Rai et al., 2022; Bidi et al., 2021; Koleva et al., 2022) (Suppl Fig. 3).

5. Conclusions

There was significant enhancement in the bamboo plants' ability to handle Hg toxicity when Fe NPs were added, both by individually and in combination with typical Fe sources like Fe S and Fe C. The data demonstrated that the utilization of Fe NPs, together with conventional Fe sources, improved the adverse effects of oxidative stress caused by Hg in bamboo plants. This improvement was achieved by enhancing antioxidant activity, antioxidant metabolism, and proline concentration. Inducing these capacities can enhance the ability to scavenge ROS, maintain membrane integrity, and preserve plant cells. Enhancement of leaf RWC improved photosynthetic efficiency, ultimately leading to the growth and development of plants exposed to Hg toxicity. Furthermore, we noticed a substantial rise in polyamines when using Fe NPs as well as other conventional Fe sources. This improvement can be linked to two key mechanisms. Firstly, there was an upgrade in nutritional availability, which led to a decrease in the accumulation and movement of Hg inside the bamboo organs. Secondly, there was a reduction in the process of Hg immobilization. Additionally, a possible approach to improve the defense mechanisms of plant cells is to enhance their antioxidant and non - antioxidant capabilities. Our findings indicate that the application of Fe NPs, either alone or in combination with iron carbide (Fe C) and iron sulfide (Fe S), has a substantial favorable impact on improving the bamboo plant's ability to withstand Hg toxicity. Potential avenues for future progress in the examined area of study, further research might be undertaken to investigate the effects of varying iron concentrations on the reduction of different types of heavy metals in bamboo species. In addition to the potential of comparing the mechanisms involved in bamboo with those of other plant species to identify the most effective amounts of Fe in mitigating metal toxicity.

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CRediT authorship contribution statement

Yang Li: Writing – review & editing, Software, Methodology, Formal analysis, Data curation. **Moxian Chen:** Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Necla Pehlivan:** Writing – review & editing, Writing – original draft, Visualization, Validation, Formal analysis, Data curation, Conceptualization. **Guohua Liu:** Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Abolghassem emamverdian:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Ahlam Khalofah:** Writing – original draft, Validation, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of Competing Interest

The authors declare no conflict of interest

Data Availability

Data will be made available on request.

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Ethics approval

Not applicable

Consent to participate

All authors agreed to contribute to this study

Consent for publication

Not applicable

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.116431.

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