The Investigation of TiO$_2$ NPs Effect as a Wastewater Treatment to Mitigate Cd Negative Impact on Bamboo Growth

Abolghassem Emamverdian$^{1,2}$, Yulong Ding$^{1,2,*}$, Farzad Mokhberdoran$^1$, Zishan Ahmad$^{1,2}$ and Yinfeng Xie$^{1,3}$

1 Co-Innovation Center for Sustainable Forestry in Southern China, NO.159, Londpan Road Nanjing, Nanjing Forestry University, Nanjing 210037, China; emamverdiyan@yahoo.com (A.E.); mfarzad649@hotmail.com (F.M.); ahmad.lycos@gmail.com (Z.A.); xxyyff@njfu.edu.cn (Y.X.)
2 Bamboo Research Institute, Nanjing Forestry University, Nanjing 210037, China
3 College of Biology and the Environment, Nanjing Forestry University, Nanjing 210037, China
* Correspondence: ylding@vip.163.com

Abstract: The recent emerging evidence reveals that titanium dioxide nanoparticles (TiO$_2$ NPs) can be used as a wastewater treatment. This study provides new information about the possible detoxification role of TiO$_2$ NPs as a wastewater treatment in plants under heavy metal stress, with an emphasis on the mechanisms involved. Here, we investigated the effects of TiO$_2$ NPs as one wastewater treatment on a bamboo species (Arundinaria pygmaea L.) under in vitro Cadmium (Cd) toxicity conditions. A factorial experiment was conducted in a completely randomized design with four replications of four concentrations of Cd (50, 100, 200, and 300 µM) alone and in combination with 100 and 200 µM TiO$_2$ NPs as two wastewater treatments, as well as a control treatment. The results indicated that TiO$_2$ NPs concentrations enhanced enzymatic and non-enzymatic antioxidant activities and proline accumulation as well as reducing hydrogen peroxide (H$_2$O$_2$), superoxide radical (O$_2^-$), and malondialdehyde (MDA) levels, which led to improved photosynthetic parameters with an eventual increase in plant biomass as compared to the control treatment. Therefore, TiO$_2$ NPs improved the photosynthetic parameters of bamboo under Cd toxicity, which led to an increase in plant biomass. We concluded that the wastewater treatments of TiO$_2$ NPs improved bamboo biomass through the scavenging of reactive oxygen species (ROS) compounds (H$_2$O$_2$ and O$_2^-$), which was induced by the stimulation of the antioxidant capacity of the plant. TiO$_2$ also protected cell membranes by reducing lipoperoxidation in bamboo under Cd toxicity. The concentration of 200 µM TiO$_2$ NPs had the most impact in reducing Cd toxicity.

Keywords: wastewater treatment; titanium dioxide nanoparticles; bamboo species; cadmium

1. Introduction

Cadmium (Cd) is deemed as one of the most toxic metals in the urban and agricultural soils of China, deleteriously affecting human health and crop production across the different regions in the country [1,2]. The major release points of Cd into the soil in China include mineral and extractive metallurgical processes, industrial and household waste disposal, excessive synthetic chemical inputs in agriculture, and aquaculture production as well as factory and automobile exhaust fumes [3,4]. Cd in plant roots alters the balance of macronutrients and micronutrients and inhibits root elongation [5]. In shoots and leaves, Cd causes a limitation in the growth and development of plants by decreasing the content of photosynthetic pigments and increasing H$_2$O$_2$ accumulation, leading to oxidative stress by ROS generation [6–10]. ROS compounds include non-radical molecules and free radical molecules such as hydroxyl radicals (•OH), hydrogen peroxide (H$_2$O$_2$), singlet oxygen (O$_2^+$), and superoxide anions (O$_2^{•−}$) [11]. These molecules can damage plant cells by increasing lipid peroxidation, accelerating protein oxidation, destroying nucleic acids, and reducing enzyme activity, which eventually leads to the death of the cells [12,13].
On the other hand, protective enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) act as scavenging ROS compounds in plant cells [14]. Hence, the exploration of new means to allay oxidative stress caused in plants by heavy metals such as Cd is of critical priority for researchers.

Nanoparticles (NPs) are small particles of less than 100 nm and are used in diverse industries such as food and food packaging, medicine, and bioremediation [15,16], and water and wastewater treatments [17]. However, in the fields of wastewater treatments, the use of nanoparticles is novel and requires further study. Moreover, the knowledge of the precise function of nanoparticles at the physiological–molecular level of the abiotic-stressed plants still remains limited [18]. There is also no comprehensive understanding of the role of engineered nanoparticles in plant physiology at the molecular level [18]. However, the physiological impacts of nanoparticles are known to vary depending on the type, size, and concentration of the nanomaterial, as well as plant species [19]. Titanium dioxide nanoparticles (TiO₂ NPs) create a nontoxic white pigment that has been used as an applied material [20]. The reason to use as wastewater treatment can be a high photocatalytic activity, the ability of photostability, economic price, and biological and chemical stability [21,22]. Recently, plant physiologists have considered the use of titanium dioxide nanoparticles because of their biological properties [23,24]. There are some studies showing the impacts of TiO₂ NPS on the various growth and development parameters of plants, including biomass and protein content, oxygen, and total nitrogen [25], as well as chlorophyll and photosynthesis properties such as electron transfer and light energy [26]. Zheng et al. (2005), in an experiment on the effects of nanoparticle and non-nanoparticle titanium dioxide on the seedling growth of spinach, concluded that in 30 days, titanium dioxide nanoparticles increased chlorophyll-a contents and dry weight by 45% and 73%, respectively, in comparison with a control. They also reported that titanium dioxide nanoparticles increase the photosynthetic rate by three-fold compared to that in control treatments [27]. Additionally, the adverse impacts of TiO₂ NPs on seeds during the germination stage have been reported [28]. It has been recently shown that TiO₂ NPs can ameliorate cadmium toxicity in soybean [29]. However, the impact of TiO₂ NPs on cadmium toxicity in bamboo plants is unknown. Therefore, investigating the possibility of reducing the toxicity of heavy metals in plants by TiO₂ NPs and identifying the mechanisms involved in toxicity reduction can be of great help in understanding the effects of TiO₂ NPs on bamboo. To our knowledge, this is the first report that explores the role of varying levels of TiO₂ NPs in the interaction with different Cd concentrations in the simultaneous eliciting of enzymatic and non-enzymatic, photosynthetic as well as growth responses of bamboo plant.

Bamboo is a crucial forest resource that plays an essential role in the livelihood of local households in southern China [30,31]. According to the 2012 report by the state forestry administration of China, the bamboo industry in the country exceeded US$19.7 billion [32]. Bamboo (Bambusoideae), with more than 70 genera and 500 species, Ref. [33] covers more than 6 million hectares of forestland in China [30]. However, in the recent decades, anthropogenic activities have increased soil contamination by heavy metals in the agricultural forestlands of southern China [34]. As a result, the bamboo plants are more exposed to heavy metal poisoning than before. Nevertheless, there is a lack of sufficient studies on reducing the toxicity of heavy metals to bamboo plants in these areas. Bamboo (Arundinaria pygmaea) is a local species in Jiangsu province of China, which has been used for landscape purposes; however, its normal growth has been influenced by heavy metals toxicity, particularly by Cd. The aim of this research was to investigate the capability of two varying concentrations of titanium dioxide nanoparticles as a wastewater treatment in ameliorating cadmium toxicity, with an emphasis on the mechanisms involved in reducing the toxic effects of cadmium, including scavenging ROS compound and amplification of plant antioxidant defense capacity.
2. Materials and Methods

2.1. Plant Materials

The experiment of tissue culture was performed using the bamboo species *Arundinaria pygmaea*, obtained from the bamboo research Institute at Nanjing Forestry University, where it has been growing since 1982. For this purpose, the nodal parts of 10 mm collected from a branch of a one-year-old of the same clone were used for the tissue culture trial in May 2017. The axillary buds on the node were induced and then proliferated. The proliferation of young shoots led to the induction of roots. Murashige and Skoog (MS) medium [35] containing 1 L of major salts (macronutrients) and 1 L of minor salts (micronutrients) was employed to set up explants culture. The medium was supplied with 0.5 mL kinetin (KT) as a growth regulator and 4 mL 6-benzyl amino purine (6-BA) in which 7–10 g of agar with 30 g sucrose were added.

2.2. Characterization of Wastewater Treatment of TiO$_2$ NPs

The TiO$_2$ NPs as a wastewater treatment were supplied from Nanjing Jiancheng Company in Jiangsu Province, China. The purity of TiO$_2$ NPs was $\geq 99.5\%$. The TiO$_2$ NPs were in the white powder form, <30 nm size.

2.3. Heavy Metal Range Concentration

The heavy metal concentrations were selected based on the previous studies by our research team where the bamboo species tolerance range to heavy metals was determined.

2.4. Experimental Design and Growth Conditions

The treatments were four concentrations of Cd (CdCl$_2$) (50, 100, 200, and 300 µM) alone and in combination with 100 or 200 µM TiO$_2$ NPs as two wastewater treatments, with four replicates and a control treatment. The experiment was carried out under controlled conditions in a plant tissue culture chamber for 30 days. After preparing 1 L of MS medium (0.5 mL kinetin (KT), 4 mL 6-benzylaminopurine (6-BA), and 30 g/L sucrose), the TiO$_2$ concentrations in combination with different concentrations of Cd were added, and the solution was adjusted to a pH of 5.8. At the final step, an optimum amount of agar (7–10 g/L agar) was added to the solution. Microwave heating was used for ten minutes to increase the solubility of the solution, and then, the solution was transferred to an autoclave for sterilization (HiClave HVE-50).

Then, 100 mL culture medium was used in a glass petri dish (60 mm diameter and 90 mm height). Afterwards, each treatment of bamboo plants cultured inside them in the ultraviolet sterilized incubation hood (Air Tech). The incubation hood consisted of white fluorescent lamps (wavelength 350–750 nm) with 25 °C. The bamboo plants, after the incubation, preserved in a controlled plant tissue culture chamber with white fluorescent lamps (wavelength 350–750 nm) with temperatures of 30/25 and 17/22 °C during light and dark periods, respectively, and a photoperiod of 16 h for 25 days. This growth condition resembled the natural habitat where the bamboo plants typically grow (Figure 1).
Figure 1. Bamboo species (Arundinaria pygmea) as affected by different Cd concentrations (0, 50, 100, 200, and 300 µM) in combination with 100 and 200 µM TiO₂NPs application levels.

2.5. Measured Indicators

The effect of the supplementation of TiO₂ NPs on the bamboo shoot development was evaluated through a set of indicators described in the following. All antioxidant enzyme activities were measured carefully, including the activities of peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), and glutathione (GSH). Additionally, hydrogen peroxide (H₂O₂), superoxide (•O⁻²), and malondialdehyde (MDA) levels were estimated. Then, for the determination of plant photosynthesis, chlorophyll indexes (total chlorophyll, chlorophyll-a, chlorophyll-b, and carotenoid contents) were measured. Ultimately, the bamboo biomass was recorded as the dry weight (DW) of the shoot and the root.

2.6. The Method of Sampling

For pre-experiment, a quantity of 0.5 g was taken from all the samples and exposed to liquid nitrogen. The samples were crushed to powder in a mortar and pestle and kept in a...
test tube at 2.8 °C. In the next step, 2 mg phosphate buffer (pH = 7.8) was added, and the samples were subjected to 20 min of centrifugation at optimum speed (2000–3000 RPM), which was necessary for supplement separation.

2.7. Determination of Antioxidant Activities

The activity of superoxide dismutase (SOD, EC 1.15.1.1) as an antioxidative enzyme was determined by the method of Zhang (1992) [36] via photoreduction in nitro blue tetrazolium (NBT). For this purpose, the sample (0.1 mL) was added to a tube containing NBT (0.2 mL), MET (0.2 mL), EDTA (0.2 mL), Rib (0.2 mL), and pH 7.0 buffer (3.1 mL). Then, the test tube was transferred to a light chamber for 10–20 min. The light exposure led to color change in the solution. In the final step, a spectrophotometer machine was used to calculate SOD value of samples.

POD (POD, EC. 1.11.1.7) activity was measured by Zhang’s method [36]. For this purpose, 0.2 mL of H$_2$O$_2$, 4 mL of 2-methoxyphenol, and 0.8 mL of pH 7 buffer solution were added to 20 mL of the sample. After preparing the supernatant, it was transferred to a spectrophotometer, and POD activity was measured according to the difference in absorbance at 470 nm.

Catalase (CAT, EC 1.11.1.6) activity was measured from two H$_2$O$_2$ reactions analyzed at 240 nm by Aebi’s method [37]. A 0.1 mL sample was added to a test tube containing 1.6 mL water, 0.2 mL H$_2$O$_2$, and 1 mL Tris-HCl. For the determination of CAT activity, the sample was transferred to a spectrometer machine (Beijing Purkinje TU-1810 UV-vis Spectrometer) and measured at 230 nm two or three times.

To determine glutathione reductase (GR), the commercial chemical assay kits provided by the Nanjing Jiancheng Company were used. After preparation, the samples were added to 0.1 mm EDTA, 2% PVP, and 0.5% (w/v) Triton-100. Then the obtained mixture was centrifuged at 10,000 RPM under an optimum temperature of 4 °C for 10 min. Then the resultant mixture was transferred to a spectrometer machine (Beijing Purkinje TU-1810 UV-vis Spectrometer). In this experiment, the glutathione reductase activity (GR) was estimated based on the manufacturer’s instructions.

The quantification of enzyme activity of ascorbate peroxidase (APX, EC 1.11.1.11) was assessed based on the method of Nakano and Asada (1981) [38]. In this experiment, 400 µL 0.5 mM ascorbic acid, 100 µL enzyme extract, and 600 µL 0.1 mM EDTA in 0.05 M Na-phosphate with (pH = 7.0) were added to a test tube. Then, to measure the reaction, 400 µL of 3% H$_2$O$_2$ was added, and the decrease in absorbance at 290 nm was calculated after 1 min. At the end of the experiment, APX activity was estimated with an extinction coefficient (€ = 2.8 mM$^{-1}$ cm$^{-1}$).

2.8. Determination of GSH and Proline Concentrations

The GSH concentration was determined according to (Ellman, 1959) [39]. For this purpose, shoot samples (0.1 g) were used. The bamboo samples were placed in 3 mL of 0.5 mM EDTA solution comprising 3% TCA at the temperature of 4 °C. The samples were then centrifuged at 15,000 × g for 15 min. Then, the obtained supernatant (0.2 mL) was mixed with 1.5 mL of 50 mM potassium phosphate buffer (pH = 7.0), including 0.2 mM of 5,5-dithiol-bis (2-nitrobenzoic) (DTNB). The incubated process was conducted on a provided solution at 30 °C for 2 min. To assessed GSH activity, the absorbance was recorded at 412 nm by a spectrometer.

The proline (pro) concentration was assessed based on the method of [40], which is conducted according to the reaction of proline with ninhydrin. For this method, shoot samples (0.2 g) were added to 3% (w/v) sulfoalicylic acid, and the mixture was centrifuged at 4000 × g for 15 min. The supernatant was used to determining proline content based on the reaction of the proline with ninhydrin.
2.9. Determination of Hydrogen Peroxide (H$_2$O$_2$), Superoxide Radicals (O$_2^{•−}$), and Malondialdehyde (MDA)

The content of hydrogen peroxide (H$_2$O$_2$) was determined by a chemical reaction where a commercial assay kit provided by (Nanjing Jiancheng Company, Nanjing, Jiangsu, China) was used. According to this method, liquid nitrogen (LN2) was used to submerge and maintain samples at the temperatures (~80 or ~20 °C) for seven days. There was a resulting loss of 60% of the H$_2$O$_2$ during the seven days. The samples, after removal from the LN2, were analyzed by speedy weighing without thawing. The samples were squashed under LN2 with a mortar and pestle. In the final step, the method of modified ferrous ammonium sulfate/xylene orange (FOX) was employed to determine the content of H$_2$O$_2$ in the extracts.

The superoxide radical (O$_2^{•−}$) content was determined according to Li’s method [41]. For this purpose, 200 mg samples of leaf tissue were homogenized in a containing phosphate buffer (65 mM, pH = 7.8) and then centrifuged at 4000 × g for 20 min. The obtained supernatant was then added to 10 mM hydroxylamine hydrochloride and optimal phosphate buffer (65 mM, pH = 7.8), which was incubated for 20 min at 25 °C. Then, the obtained solution was mixed with 7 mM α-naphthylamine and 17 mM sulfanilamide. The obtained supernatant after resting for 20 min was transferred to the spectrometer machine for the determination of the O$_2^{•−}$ content at 530 nm at 25 °C. The generation rate of O$_2^{•−}$ was quantified by standard curve with nitrogen dioxide radical (NO$_2$).

Malondialdehyde (MDA) levels, as an indicator of lipid peroxidation, were quantified by using a reaction of thiobarbituric acid (TBA) with Kai and Feng’s method [42]. According to the method, 10% trichloroacetic acid (TCA) was used to homogenize 0.5 g of samples, which were then centrifuged at 4000 × g for 15 min. The obtained supernatant was recorded to determine the MDA content. For this purpose, the final supernatant (2 mL) and 2 mL of 0.6% TBA were exposed to 100 °C for 20 min and then instantaneously cooled in an ice bath. As the last step, the solution was centrifuged at 4000 × g for 15 min and transferred to a spectrometer machine for the measurement of the absorbance at 450, 532, and 600 nm.

2.10. Determination of Total Chlorophyll, Chlorophyll-a, Chlorophyll-b, and Carotenoids

The contents of chlorophyll-a, chlorophyll-b, and carotenoid were determined based on Arnon’s method [43]. For this purpose, leaf sample (0.5 g) was dissolved in a porcelain mortar, crushed with liquid nitrogen, and pulverized. In the next step, 20 mL 80% acetone at 0 to 4 °C was added to the sample-containing test tubes. The centrifugation of the samples occurred at 6000 RPM for 10 min. Then, the obtained supernatant was poured into the glass balloon and transferred to the spectrophotometer machine for the final step. The absorbance spectra of the samples were measured by spectrophotometer with a wavelength of 663, 645, and 470 nm, for chlorophyll-a, chlorophyll-b, and carotenoid content, respectively. To determine the amount of chlorophyll-a, chlorophyll-b, and carotenoids (mg/g fresh weight), the following formulas were used:

\[
\text{Chlorophyll-a} = (A_{663} \times 19.3 - A_{645} \times 0.86) V/100W
\]

\[
\text{Chlorophyll-b} = (A_{645} \times 19.3 - A_{666} \times 33.6) V/100W
\]

\[
\text{Carotenoids} = (A_{470}) 100 - 104 (\text{mg chl. b}) - 3.27 (\text{mg chl. a})/227
\]

where, V = the volume for the filtered solution (The resultant supernatant after centrifugation); W = fresh weight of sample (g); and A = absorbance at 470, 645, or 663 nm.

2.11. Biomass Measurements

In the last step of the experiment, after TiO$_2$ NPs-Cd exposure, the bamboo shoots and roots were carefully clean and washed. A vacuum drying oven (DZF-6090) was used to dry...
the water from the plant surface. The samples were kept at 110 °C for 15 min, and then the samples were dried to a constant dry weight at 80 °C. The dry weight in this experiment was determined from the root and shoot biomass and was measured for four replicates.

2.12. Statistical Analysis

In this study, the statistical software package R was used to perform data analysis. The experiment was carried out in a completely randomized design (CRD) with four replicates, and the results were analyzed using two-way ANOVA. To determine the mean differences, Tukey’s test was used at the probability level $p < 0.05$.

3. Results

3.1. The Impact of Cd and TiO$_2$ NPs on Various Antioxidant Enzyme Capacities

The results obtained through analysis of the data on the activities of antioxidant enzymes (SOD, CAT, POD, GR, and APX) indicated that there was a significant difference in antioxidant enzyme activity among the various concentrations of Cd and TiO$_2$ NPs in *Arundinaria pygmea* ($p < 0.001$). Figure 2 shows that excess heavy metals reduced the antioxidant activity, as the levels of 200 µL and 300 µL Cd resulted in the lowest antioxidant activities. However, it was clear that the combination of Cd with TiO$_2$ NPs significantly improved all the antioxidant activities in the bamboo species. According to the results, the highest antioxidant activities were found at 200 µL TiO$_2$ NPs in combination with 50 µL Cd for SOD, POD, CAT, and APX; their activities were 1.79, 1.85, 1.97, and 2 times those in the control, respectively. Additionally, 200 µL TiO$_2$ showed a 2.32-fold enhancement in GR activity compared with those in the control treatment. These results showed the ability of 200 µL TiO$_2$ to stimulate antioxidant activities. On the other hand, the results indicated that treatment with 300 µL Cd led to the lowest antioxidant activities in the bamboo species where there were reductions in SOD, POD, CAT, GR, and APX contents by 80%, 65%, 70%, 69%, and 53%, respectively, as compared with those of the control. However, in this study, the TiO$_2$ treatments (100 and 200 µL) indicated that TiO$_2$ had significant potential to increase plants’ antioxidant capacity under Cd toxicity, as shown in Figure 2. In general, as shown in Table 1, the results indicated that the highest values of GR, CAT, POD, SOD, and APX activities in the bamboo species under Cd stress were 74%, 48%, 47%, 45%, and 41.5%, respectively, which were higher than those in the control treatment.

![Figure 2. Cont.](image-url)
Figure 2. Effects of TiO$_2$ NPs concentrations on antioxidant enzyme activities (A–E), GSH (F), and proline concentrations (G) of *Arundinaria pygmea* under different concentrations of Cd. The treatments included different concentrations of Cd alone or in combination with various levels of TiO$_2$ NPs (100 and 200 µM). The capital letters indicate statistically significant differences across different concentrations of Cd treatment alone or in combination with TiO$_2$NPs (the bars with the same colors), while the lowercase letters indicate statistically significant differences within each concentration of Cd treatment alone or in combination with TiO$_2$NPs (the bars with different colors) according to Tukey’s test ($p < 0.05$).
### Table 1. The percentage of change in antioxidant enzymatic activities, H$_2$O$_2$, and MDA contents under the different concentrations of Cd- (TiO$_2$ NPs) compared to their control treatments. The arrows show the percentage reduction of indexes.

| Concentration of Cd-(TiO$_2$NPs) Combination | SOD | POD | CAT | APX | GR | GSH | Proline | H$_2$O$_2$ | O$_2^{•−}$ | MDA |
|---------------------------------------------|-----|-----|-----|-----|----|-----|---------|-----------|----------|------|
| 0 × 100 μM                                  | 2%  | 17% | 1.10% | 6.50% | 28% | 18% | 26%     | 46% ↓     | 23% ↓    | 16% ↓|
| 0 × 200 μM                                  | 51% | 51% | 47%  | 74%  | 132% | 40% | 79%     | 72% ↓     | 50% ↓    | 54% ↓|
| 50 × 100 μM                                 | 40% | 63% | 23%  | 50%  | 13% | 51% | 46%     | 29% ↓     | 29% ↓    | 24% ↓|
| 50 × 200 μM                                 | 79.10% | 85% | 97%  | 100% | 71% | 102% | 126%    | 73% ↓     | 54.5% ↓  | 64% ↓|
| 100 × 100 μM                                | 6%  | 4% ↓| 1.40% | 11%  | 27% | 49% | 44%     | 20% ↓     | 18% ↓    | 32% ↓|
| 100 × 200 μM                                | 74% | 38% | 80%  | 48.50% | 81% | 73% | 107%    | 63% ↓     | 50.5% ↓  | 42% ↓|
| 200 × 100 μM                                | 8% ↓| 3%  | 2% ↓| 16%  | 55% | 57% | 38%     | 28% ↓     | 4% ↓     | 20% ↓|
| 200 × 200 μM                                | 63% | 42% | 84%  | 80%  | 102% | 92% | 105%    | 50% ↓     | 24% ↓    | 40% ↓|
| 300 × 100 μM                                | 18% | 62% | 10%  | 4%   | 106% | 56% | 94%     | 16% ↓     | 14% ↓    | 14% ↓|
| 300 × 200 μM                                | 75% | 63% | 85%  | 27%  | 129% | 73% | 97%     | 41% ↓     | 19% ↓    | 39% ↓|

#### 3.2. The Impact of Cd and TiO$_2$ NPs on GSH and Proline Concentrations

The results obtained by analyzing the GSH activity and proline accumulation data indicated that there were significant differences among the various levels of TiO$_2$ and Cd and the control treatment (p < 0.001). According to the results, GSH activity and proline concentration decreased with excess Cd concentrations, while the combination of TiO$_2$ and Cd showed a significant positive impact on the GSH activity and proline accumulation in the bamboo under the different Cd concentrations. The highest values for GSH activity and proline accumulation were found in the treatments with 200 μL TiO$_2$–50 μL Cd, with 2.02- and 2.26-fold increases in GSH activity and proline accumulation, respectively. The 300 μL Cd treatment showed the lowest levels of GSH activity and proline concentration (0.55 μmol g$^{-1}$ FW and 1.87 μg g$^{-1}$ FW, respectively).

#### 3.3. The Impact of Cd and TiO$_2$ NPs on Hydrogen Peroxide (H$_2$O$_2$), Superoxide Radicals (O$_2^{•−}$), and Lipid Peroxidation (MDA)

In this study, the effects of TiO$_2$ on ROS accumulation and the peroxidation of lipid membranes were investigated. The data analysis showed that the various Cd and TiO$_2$ NPs treatments had a significant effect (p < 0.001) on hydrogen peroxide (H$_2$O$_2$) and superoxide radical (O$_2^{•−}$) accumulation. They showed an increasing trend with excess heavy metal concentrations. However, the results indicated that TiO$_2$ NPs could ameliorate the Cd-induced toxicity and consequently reduce oxidative stress caused by the ROS compounds (H$_2$O$_2$ and O$_2$). The results indicated that the greatest reduction of H$_2$O$_2$ and O$_2^{•−}$ was found in the 200 μL TiO$_2$ + 50 μL Cd treatments, with 1.72- and 1.54-fold reductions compared with the control, respectively. These results indicate that 200 μL TiO$_2$ is the most effective for amelioration of the damaging impacts of the ROS compounds. However, 100 μL TiO$_2$ NPs reduced hydrogen peroxide (H$_2$O$_2$) and superoxide radical (O$_2^{•−}$) levels in the bamboo species under the various concentrations of Cd. In the present research, the greatest increase in H$_2$O$_2$ and O$_2^{•−}$ was attributed to 300 μL Cd, with 102% and 68% increases compared to the control, respectively, so the excess of Cd led to the generation of oxidative stress caused by ROS in our bamboo species.

The data analysis showed that the various concentrations of Cd-(TiO$_2$ NPs) had significant effects (p < 0.001) on the lipid peroxidation (MDA) level. Figure 3 shows that 200 μL TiO$_2$–50 μL Cd had the highest impact on the reduction of MDA in the bamboo species, with a 64% reduction compared with the control. This reveals the protective role of TiO$_2$ NPs on the cell membrane and membrane structures. In general, as shown in Table 1, the combination of TiO$_2$ NPs with Cd can reduce the damaging effect of Cd on cells and cell membranes. Figure 3 shows that TiO$_2$ NPs (100 and 200 μL) significantly
reduced lipid peroxidation (MDA) at the different concentrations of Cd, even at a high concentration of Cd. However, this protective role was less effective at high concentrations of Cd (200–300 µL). The greatest increase in lipid peroxidation (MDA) was found at the concentration of 300 µL Cd, with a 4.94-fold increase compared with that of the control treatment. This result indicates the damaging role of Cd toxicity on cell membranes and cell integrity.

Figure 3. Effects of TiO\textsubscript{2} NPs concentrations on hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) (A), superoxide radicals (O\textsubscript{2}•\textsuperscript{−}) (B), and malondialdehyde (MDA) (C) of \textit{Arundinaria pygmea} under different concentrations of Cd. The treatments included different concentrations of Cd alone or in combination with various levels of TiO\textsubscript{2} NPs (100 and 200 µM). The capital letters indicate statistically significant differences across different concentrations of Cd treatment alone or in combination with TiO\textsubscript{2} NPs (the bars with the same colors), while the lowercase letters indicate statistically significant differences within each concentration of Cd treatment alone or in combination with TiO\textsubscript{2} NPs (the bars with different colors) according to Tukey’s test (\(p < 0.05\)).

3.4. The Impact of Cd and TiO\textsubscript{2} NPs on the Contents of Total Chlorophyll, Chlorophyll-a, Chlorophyll-b, and Carotenoids

According to the data analysis, there were significant differences among the various concentrations of Cd and TiO\textsubscript{2} NPs (\(p < 0.001\)) in terms of the contents of total chlorophyll, chlorophyll-a, chlorophyll-b, and carotenoids. Table 2 shows that chlorophyll and carotenoid contents were significantly reduced by Cd. The greatest reductions in the contents of total chlorophyll (39%), chlorophyll-a (31%), chlorophyll-b (41%), and carotenoids
(29%) were observed in the treatments with 300 µL Cd in comparison with the control treatment. However, the results indicated that the addition of TiO$_2$ NPs could help to ameliorate Cd in this bamboo species, which was shown by the increasing total chlorophyll, chlorophyll-a, chlorophyll-b, and carotenoids under different combinations of Cd and TiO$_2$ NPs. Our results indicated that TiO$_2$ NPs (100 and 200 µL) could significantly reduce the damaging effect of cadmium on chlorophyll and carotenoid contents. The greatest increases in total chlorophyll, chlorophyll-a, chlorophyll-b, and carotenoids contents were found in the treatments with 200 µL TiO$_2$ NPs–50 µL Cd, with 1.37-, 1.25-, 1.62-, and 1.47-fold increases in comparison with the control, respectively.

Table 2. The effect of the combination of Cd- (TiO$_2$ NPs) on the content of chlorophylla, chlorophyllb, total chlorophyll, and carotenoids. Each data point is the mean ± SE of five replicates. The treatments included four concentrations of Cd (50, 100, 200, and 300 µM) alone and in combination with 100 or 200 µM TiO$_2$ NPs. The capital letters indicate statistically significant differences within each concentration of Cd treatment alone or in combination with TiO$_2$NPs according to Tukey’s test ($p < 0.05$).

| Cd (µM) | (TiO$_2$ NPs) | Chla (µg g$^{-1}$ F.w.) | Chlb (µg g$^{-1}$ F.w.) | T. Chl (µg g$^{-1}$ F.w.) | Carotenoids (µg g$^{-1}$ F.w.) |
|---------|---------------|-------------------------|-------------------------|---------------------------|----------------------------------|
| 0       | 0             | 6.188 ± 0.200$^\text{Ab}$ | 3.640 ± 0.204$^\text{Ab}$ | 9.828 ± 1.930$^\text{Aa}$ | 34.10 ± 1.259$^\text{Ab}$ |
| +TiO$_2$ 100 | 6.469 ± 0.371$^\text{Ab}$ | 3.966 ± 0.298$^\text{Ab}$ | 10.93 ± 1.040$^\text{Aa}$ | 35.88 ± 1.303$^\text{Ab}$ |
| +TiO$_2$ 200 | 7.124 ± 0.268$^\text{Ab}$ | 4.808 ± 0.286$^\text{Aa}$ | 11.93 ± 2.100$^\text{Aa}$ | 48.48 ± 1.674$^\text{Aa}$ |
| 50      | 0             | 5.527 ± 0.392$^\text{AAb}$ | 2.949 ± 0.333$^\text{Bc}$ | 8.72 ± 1.075$^\text{AAb}$ | 31.36 ± 1.885$^\text{Ac}$ |
| +TiO$_2$ 100 | 5.926 ± 0.573$^\text{C}$ | 3.873 ± 0.159$^\text{Ab}$ | 9.54 ± 1.079$^\text{AAb}$ | 35.12 ± 1.510$^\text{Ab}$ |
| +TiO$_2$ 200 | 6.949 ± 0.183$^\text{Aa}$ | 4.531 ± 0.199$^\text{Aa}$ | 11.48 ± 1.678$^\text{Aa}$ | 45.60 ± 1.660$^\text{Aa}$ |
| 100     | 0             | 5.188 ± 0.401$^\text{BCb}$ | 2.670 ± 0.249$^\text{Bb}$ | 8.10 ± 0.994$^\text{AAb}$ | 29.46 ± 2.721$^\text{AAb}$ |
| +TiO$_2$ 100 | 5.918 ± 0.200$^\text{AAb}$ | 3.596 ± 0.352$^\text{Aa}$ | 9.51 ± 1.355$^\text{AAb}$ | 32.75 ± 1.938$^\text{AAb}$ |
| +TiO$_2$ 200 | 6.396 ± 0.326$^\text{Aa}$ | 3.998 ± 0.161$^\text{Ba}$ | 10.89 ± 1.147$^\text{Aa}$ | 36.33 ± 1.302$^\text{Ba}$ |
| 200     | 0             | 4.574 ± 0.354$^\text{CDa}$ | 1.923 ± 0.200$^\text{Cb}$ | 6.74 ± 0.998$^\text{BCa}$ | 24.90 ± 2.507$^\text{BCb}$ |
| +TiO$_2$ 100 | 5.131 ± 0.697$^\text{BCa}$ | 2.163 ± 0.150$^\text{Bb}$ | 7.54 ± 1.004$^\text{Ba}$ | 27.02 ± 2.593$^\text{Bab}$ |
| +TiO$_2$ 200 | 5.573 ± 0.524$^\text{Ba}$ | 2.774 ± 0.142$^\text{Ca}$ | 8.34 ± 2.135$^\text{Aba}$ | 30.59 ± 1.609$^\text{Ca}$ |
| 300     | 0             | 4.205 ± 0.321$^\text{D}$ | 1.624 ± 0.190$^\text{Cb}$ | 5.33 ± 1.104$^\text{Cb}$ | 23.63 ± 2.846$^\text{Ca}$ |
| +TiO$_2$ 100 | 4.845 ± 0.381$^\text{C}$ | 2.084 ± 0.204$^\text{Ba}$ | 7.18 ± 1.055$^\text{Bab}$ | 25.59 ± 3.447$^\text{Ba}$ |
| +TiO$_2$ 200 | 5.283 ± 0.361$^\text{B}$ | 2.416 ± 0.175$^\text{Ca}$ | 7.95 ± 0.992$^\text{Ba}$ | 28.006 ± 2.699$^\text{Ca}$ |

3.5. The Impact of Cd and TiO$_2$ NPs on the Productions of Biomass in Shoots and Roots

In this study, the biomass indexes were the dry weight (DW) of the shoot and the root. According to our results, there were significant differences among the various concentrations of Cd and TiO$_2$ NPs in the DW of the shoots and the roots of bamboo ($p < 0.001$). The DW of the roots and the shoots was significantly decreased with the addition of Cd. The greatest reduction occurred at the highest concentration of Cd, with 0.23 g (56%) and 0.16 g (74%) reductions in the DW of the shoots and the roots, respectively, compared with those of the control. The results also showed that the TiO$_2$ NPs (100 and 200 µL) alone or in combination with Cd helped to increase the DW of the shoots and the DW of the roots. Figure 4 shows that the greatest increases in the DW of the shoots and the DW of the roots occurred in the 200 µL TiO$_2$ and 200 µL TiO$_2$–50 µL Cd treatments, with 0.87 g (67%) and 0.86 g (77%) increases in the dry weight of the shoots and 0.93 g (54%) and 0.89 g (57%) increases in the dry weight of the roots, respectively, compared with those under the control treatments. This indicated that 200 µL TiO$_2$ NPs was more effective than
100 µL TiO₂ NPs. However, both concentrations of TiO₂ NPs increased the plant biomass under Cd stress (Table 3).

Figure 4. Effects of TiO₂ NPs concentrations on shoot dry weight (A) and root dry weight (B) in Arundinaria pygmea under different concentrations of Cd. The treatments included different concentrations of Cd alone or in combination with various levels of TiO₂ NPs (100 and 200 µM). The capital letters indicate statistically significant differences across different concentrations of Cd treatment alone or in combination with TiO₂ NPs (the bars with the same colors), while the lowercase letters indicate statistically significant differences within each concentration of Cd treatment alone or in combination with TiO₂ NPs (the bars with different colors) according to Tukey’s test (p < 0.05).

Table 3. The rate of increase in root dry weight and shoot dry weight of bamboo species under different concentrations of Cd– (TiO₂ NPs), compared to their control treatments.

| Concentration of (TiO₂ NPs) Combination | 0 µM | 50 µM | 100 µM | 200 µM | 300 µM |
|----------------------------------------|------|------|-------|-------|-------|
|                                        | 100 µM | 200 µM | 100 µM | 200 µM | 100 µM | 200 µM | 100 µM | 200 µM | 100 µM | 200 µM |
| Shoot (fold)                           | 1.36 | 1.67 | 1.23 | 1.77 | 1.33 | 1.64 | 1.08 | 1.55 | 1.13 | 1.56 |
| Root (fold)                            | 1.11 | 1.54 | 1.14 | 1.57 | 1.06 | 1.48 | 1.11 | 1.52 | 1.23 | 1.5 |

4. Discussion

In plants, abiotic stressors such as heavy metals can often lead to oxidative stress and the inhibition of growth and development, which is mostly related to the generation of ROS compounds in plant organs [44]. Oxidative stress in plants disturbs the balance between antioxidant defenses and ROS [45]. Plants have a provision of specific defense mechanisms for dealing with stress, which includes enzymatic and non-enzymatic antioxidants. Enzymatic components include superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), catalase (CAT), peroxidase (POD), and guaiacol peroxidase (GPX), and non-enzymatic antioxidants include reduced glutathione (GSH), the osmolyte proline, ascorbic acid (AA), flavonoids, and α-tocopherol [44]. The enzymes SOD, POD, and CAT scavenge ROS through the detoxification of H₂O₂ and O₂•− and inhibit the formation of •OH radicals [5]. In the antioxidant defense system, superoxide dismutase (SOD) has been reported as the first line of enzymatic defense in coping with reactive oxygen species (ROS) [46]. SOD can convert O₂•− into H₂O₂, while ascorbate peroxidase (APX), catalase (CAT), and peroxidase (POD) catalyze H₂O₂ to H₂O [47]. APX in the ascorbate–glutathione cycle converts H₂O₂ to water using two molecules of ascorbic acid and concomitantly produces monodehydroascorbate [48]. Glutathione reductase (GR, EC, 1.6.4.2) belongs to the group of disulfide [49] and has the ability to ameliorate damaging levels of H₂O₂ through some involved mechanisms [50]. GR in the sulphydryl group of GSH reduces bonds of disulfide in glutathione, which leads to the scavenging
of ROS in plant cells [51]. GR, GSH, and APS are essential components of the ASC–GSH cycle, which can eliminate 
\( \text{H}_2\text{O}_2 \) in cell compartments [5]. Our results revealed that while increasing Cd levels reduces antioxidant activity, the combination of TiO\(_2\) NPs and Cd significantly improves plant antioxidant capacity under cadmium stress. Our results show the ability of TiO\(_2\) NPs to stimulate antioxidant defense mechanisms under metal toxicity conditions. The impact of TiO\(_2\) NPs on increasing antioxidant activities has been reported as increasing the activity of superoxide dismutase in Spirodela polyrhiza [52]; enhancing POD, SOD, and CAT activity in Lemma minor [53]; and elevating CAT and GR activities in Hydrilla verticillata [54]. The findings of these research works are in line with those obtained results in this study, indicating that TiO\(_2\) NPs have the ability to increase the antioxidant defense capacity of plants.

GSH is a tiny molecule with a low molecular weight that belongs to the thiol group of compounds. GSH, which is highly distributed in organelles, can play a crucial role in plant responses to stress by providing a buffering system, which can improve redox imbalances [55]. l-glutamate is shared by GSH and proline as a biosynthesis precursor [56,57]. Some researchers have shown that TiO\(_2\) NPs can increase GSH activity or the ratio of reduced-to-oxidized glutathione (GSH/GSSG); this has been reported in wheat (Triticum aestivum) leaves [58] and in lettuce crop [59]. This effect is confirmed by the present experiment. Our results show that TiO\(_2\) NPs could increase GSH activity in the bamboo species under toxic levels of Cd, which proves that TiO\(_2\) NPs have the ability to stimulate nonenzymatic antioxidant activity. Some studies have reported that the relationships between GSH metabolism and proline accumulation can ameliorate metal stress in plants [56,57,60,61]. The primary role of proline in the reduction of metal stress is related to the role of proline accumulation in ROS scavenging through dehydration; proline is also transferred and stored as a reductant that can protect the plant from osmotic stress [62]. Additionally, proline can increase antioxidative capacity in plants under stress conditions [63]. Proline can preserve the osmotic balance and cell turgor and can protect cell membranes by reducing electrolyte leakage [64]. In the present research, the results indicated that TiO\(_2\) NPs increased proline accumulation in the bamboo species under Cd stress, which can be a reason to induce antioxidant activities. An increase in proline from the addition of TiO\(_2\) NPs is reported by Arafat in broad beans [65].

Reactive oxygen species (ROS) include superoxide radicals (O\(_2^•^-\)), hydrogen peroxide (H\(_2\)O\(_2\)), and hydroxyl radicals (HO\(^•\)). These ROS compounds are responsible for oxidative stress in various organelles of the plant. They lead to deleterious impacts such as damage to cell compounds, lipid and protein peroxidation, DNA fragmentation, and inhibition of enzyme activity and eventual cell death [66,67]. Superoxide anion O\(_2^•^-\) is scavenged by SOD, while H\(_2\)O\(_2\) is catalyzed by GPX and CAT [9,10]. According to the results, TiO\(_2\) NPs can improve cell membranes by reducing MDA content. Therefore, TiO\(_2\) NPs have the ability to decrease cellular levels of lipoperoxidation. The decrease in ROS (H\(_2\)O\(_2\), O\(_2^•^-\)) and MDA contents by the addition of TiO\(_2\) NPs has been reported in some studies, including the reduction of H\(_2\)O\(_2\) and MDA in chickpea [68], the reduction of H\(_2\)O\(_2\) and MDA in barley shoots [69], and the reduction of MDA content in wheat roots [70].

The cell wall act as a barrier to external factors. However, nanoparticles with less than 20 nm in diameter are able to easily pass through the cell wall and enter the interior of the cell and the plasma membrane. These nanoparticles are also able to smoothly pass through stomata with the diameter range of a micron [71]. Mohammadi et al. (2013) observed titanium dioxide nanoparticles in chickpea cells using SEM [68]. Gao et al. (2008), in a study on spinach, reported that there is a correlation between titanium nanoparticles and the rate of photosynthesis that may be related to the impact of titanium nanoparticles on improving light absorption, light transmission, and light conversion; however, they
confirmed the role of titanium nanoparticles in the increased activity of Rubisco activase, which increases Rubisco carboxylation and photosynthesis rates and eventually leads to plant growth [72]. In another study on spinach seeds, TiO$_2$ NPs led to a triple increase in photosynthesis and increased chlorophyll by 45% [27]. The increase in chlorophyll contents by the addition of TiO$_2$ NPs has been reported in _L. minor_ [53], _Spinacia oleracea_ [73], and _Vetiveria zizanioides_ by L. Nash [74]. Carotenoids are involved in protecting the photosynthetic reaction centers from oxidative stress caused by abiotic stressors [75]. The results of the present research indicated that TiO$_2$ NPs increased chlorophyll contents, including total chlorophyll, chlorophyll-a, chlorophyll-b, and carotenoids in the bamboo species under Cd stress. This may be related to the role of TiO$_2$ NPs in improving light absorption, transmission, and light conversion in bamboo species. One study on _Spinacia_ reported that TiO$_2$ NPs increase the activity of chloroplasts and the Hill reaction in photosynthesis in the plant, which influences the reduction of FanCy and oxygen evolution reactions [73]. TiO$_2$ NPs, thanks to their tiny size, may move into the chloroplasts and decrease the oxidation reactions caused by ROS, leading to increasing oxygen evolution reactions and electron transport in plant photosynthesis [53].

Some studies have revealed that TiO$_2$ NPs can boost plant growth and development in different species such as maize (_Zea mays_ L.) [76] and wheat [77]. In one study on _L. minor_, the result showed that biomass indexes, including fresh weight and root length, were increased by the addition of concentrations lower than 500 mg/L of TiO$_2$ NPs [53]. Another study indicated that TiO$_2$ NPs could enhance leaf area, length of shoot, and root dry weight in broad beans [65], which could be related to the increase in photosynthetic indexes caused by TiO$_2$ NPs [65]. Some studies show that the application of TiO$_2$ NPs can regulate critical enzymatic activities (e.g., nitrate reductase activity), which can increase plant growth through the accumulation of additional nutrients in plants [78,79]. The bamboo biomass indexes in our study revealed that TiO$_2$ NPs can increase the dry weight of the shoots and the roots under cadmium stress by enhancing antioxidant activity and photosynthetic properties.

5. Conclusions

The use of nanoparticles as a wastewater treatment in plant and environmental sciences offers great potentials in terms of decreasing environmental contamination and protecting plants against various abiotic stresses. The expansion of research into this area can help in a better understanding of the role of nanoparticles as wastewater treatment for detoxification purposes. In recent years, the use of titanium dioxide nanoparticles has been considered by many researchers because of their biological characteristics and more importantly possible detoxification effects on plants. Cadmium is one of the most hazardous heavy metals in China’s agricultural and forest lands. It seems that TiO$_2$ NPs, a nontoxic white pigment with a particle size of <30 nm, can play an important role in the amelioration and reduction of cadmium toxicity. In the current research, the impacts of various concentrations of TiO$_2$ NPs as one wastewater treatment on the amelioration of Cd toxicity in a bamboo species were investigated. Bamboo can be considered as an environmentally friendly plant, which can be used in phytoremediation technologies to clean up toxins from contaminated soil and water. In the present work, TiO$_2$ NPs as a wastewater treatment could play a beneficial role in improving the bamboo plant growth under Cd toxicity, which can contribute to reduction of contamination in the environment.

**Author Contributions:** Statistical analysis: A.E., Y.D., F.M., Z.A. and Y.X.; writing—original draft preparation: A.E., Y.D., F.M., Z.A. and Y.X.; investigation: A.E. and Y.D.; supervision: Y.D.; project administration: Y.D.; funding acquisition: Y.D. and A.E. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by the financial support provided by Nanjing Forestry University (Start-Up Research Fund) and Bamboo Research Institute for the current study. Special Fund for this work was supported by National Key Research and Development Program of China (Integration
and Demonstration of Valued and Efficiency–Increased Technology across the Industry Chain for Bamboo, 2016 YFD0600901).

**Institutional Review Board Statement:** This study conducted on bamboo plant at Bamboo Research Institute, Nanjing Forestry University and doesn’t need any approval statement or license.

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare that there is no conflict of interests regarding the publication of this paper.

**References**

1. Qin, Q.; Li, X.; Wu, H.; Zhang, Y.; Feng, Q.; Tai, P. Characterization of cadmium (108Cd) distribution and accumulation in Tagetes erecta L. seedlings: Effect of split-root and of remove-xylem/phloem. *Chemosphere* 2013, 93, 2284–2288. [CrossRef] [PubMed]
2. Yu, R.; He, L.; Cai, R.; Li, B.; Li, Z.; Yang, K. Heavy metal pollution and health risk in China. * Glob. Health J.* 2017, 1, 47–55. [CrossRef]
3. Wang, L.; Cui, X.; Cheng, H.; Chen, F.; Wang, J.; Zhao, X.; Lin, C.; Pu, X. A review of soil cadmium contamination in China including a health risk assessment. *Environ. Sci. Pollut. Res.* 2015, 22, 16441–16452. [CrossRef] [PubMed]
4. Sun, M.; Wang, T.; Xu, X.; Zhang, L.; Li, J.; Shi, Y. Ecological risk assessment of soil cadmium in China’s coastal economic development zone: A meta-analysis. *Ecosyst. Health Sustain.* 2020, 6, 1. [CrossRef]
5. Serrano, R.M.; Puertas, R.M.C.; Zabalza, A.; Corpas, F.J.; Gomez, M.; Del Rio, L.A.; Sandalio, L.M. Cadmium effect on oxidative metabolism of pea (Pisum sativum L.) roots. Imaging of reactive oxygen species and nitric oxide accumulation in vivo. *Plant Cell Environ.* 2006, 29, 1532–1544. [CrossRef]
6. Nowak, H.B.; Dresler, S.; Matraszek, R. Exogenous malic and acetic acids reduce cadmium phytotoxicity and enhance cadmium accumulation in roots of sunflower plants. *Plant Physiol. Biochem.* 2015, 94, 225–234. [CrossRef] [PubMed]
7. Kefaloyianni, E.; Gourgou, E.; Ferle, V.; Kotsakis, E.; Gaitanaki, C.; Beis, I. Acute thermal stress and various heavy metals induce tissue-specific pro- or anti-apoptotic events via the p38-MAPK signal transduction pathway in Mytilus galloprovincialis (Lam.). *J. Exp. Biol.* 2005, 208, 4427–4436. [CrossRef]
8. Gill, S.S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* 2010, 48, 909–930. [CrossRef]
9. Li, Y.; Li, M.; Shi, J.; Yang, X.; Wang, Z. Hepatic antioxidative responses to PCDPSs and estimated short-term bio-toxicity in freshwater fish. *Aquat. Toxicol.* 2012, 120, 90–98. [CrossRef]
10. Stegeman, J.J.; Brouwer, M.; Di Giulio, R.T.; Forlin, L.; Fowler, B.A. Enzyme and protein synthesis as indicator of contaminant exposure and effect. In *Biomarkers: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress*; Huggett, R.J., Ed.; SETAC Special Publication Series; Lewis Publishers: Chelsea, MI, USA, 1992; pp. 235–335.
11. Heyno, E.; Mary, V.; Schopfer, P.; Liszkay, K.A. Oxygen activation at the plasma membrane: Relation between superoxide and hydroxyl radical production by isolated membranes. *Planta* 2011, 234, 35–45. [CrossRef]
12. Srivastava, S.; Dubey, R.S. Manganese-excess induces oxidative stress, lowers the pool of antioxidants and elevates activities of key antioxidative enzymes in rice seedlings. *Plant Growth Regul.* 2011, 64, 1–16. [CrossRef]
13. Sharma, P.; Dubey, R.S. Drought Induces Oxidative Stress and Enhances the Activities of Antioxidant Enzymes in Growing Rice Seedlings. *Plant Growth Regul.* 2005, 46, 209–221. [CrossRef]
14. Sytar, O.; Kumar, A.; Latowsky, D.; Kuczynska, P.; Strzałka, K.; Prasad, M.N.V. Heavy metal-induced oxidative damage, defense reactions, and detoxification mechanisms in plants. *Acta Physiol. Plant.* 2013, 35, 985–999. [CrossRef]
15. Roco, M.C.; Mirkin, C.A.; Hersam, M.C. Nanotechnology research directions for societal needs in 2020: Summary of international study. *J. Nanoparticle Res.* 2011, 13, 897–919. [CrossRef]
16. Gui, X.; Zhang, Z.; Liu, S.; Ma, Y.; Zhang, P.; He, X.; Li, Y.; Zhang, J.; Li, H.; Rui, Y.; et al. Fate and Phytotoxicity of CeO2 Nanoparticles on Lettuce Cultured in the Potting Soil Environment. *PLoS ONE* 2015, 10, e4261. [CrossRef]
17. Lu, H.; Wang, J.; Stoller, M.; Wang, T.; Bao, Y.; Hao, H. An Overview of Nanomaterials for Water and Wastewater Treatment. *Adv. Mater. Sci. Eng.* 2016, 1, 1–10. [CrossRef]
18. Siddiqui, M.H.; Whalbi, A.M.H. Role of nano-SiO2 in germination of tomato (Lycopersicum esculentum seeds Mill.). *Saudi J. Biol. Sci.* 2014, 21, 13–17. [CrossRef]
19. Gao, J.; Xu, G.; Qian, H.; Liu, P.; Zhao, P.; Hu, Y. Effects of nano-TiO2 on photosynthetic characteristics of Ulmus elongata seedlings. *Environ. Pollut.* 2013, 176, 63–70. [CrossRef]
20. Skocaj, M.; Filipic, M.; Petkovic, J.; Novak, S. Titanium dioxide in our everyday life; is it safe? *Radiol. Oncol.* 2011, 45, 227–247. [CrossRef]
21. Guesh, K.; Mayoral, Á.; Álvarez, M.C.; Chebude, Y.; Díaz, I. Enhanced photocatalytic activity of TiO2 supported on zeolites tested in real wastewaters from the textile industry of Ethiopia. *Microporous Mesoporous Mater.* 2016, 225, 88–97. [CrossRef]
22. Rawal, S.B.; Bera, S.; Lee, D.; Jang, D.J.; Lee, W.I. Design of visible-light photocatalysts by coupling of narrow bandgap semiconductors and TiO$_2$: Effect of their relative energy band positions on the photocatalytic efficiency. Catal. Sci. Technol. 2013, 3, 1822–1830. [CrossRef]

23. Qi, M.; Liu, Y.; Li, T. Nano-TiO$_2$ Improve the Photosynthesis of Tomato Leaves under Mild Heat Stress. Biol. Trace Element Res. 2013, 156, 323–328. [CrossRef] [PubMed]

24. Faraz, A.; Faizan, M.; Fariduddin, Q.; Hayat, S. Response of Titanium Nanoparticles to Plant Growth: Agricultural Perspectives. Sustain. Agric. Rev. 2020, 41, 101–110. [CrossRef]

25. Yang, F.; Liu, C.; Gao, F.; Su, M.; Wu, X.; Zheng, L.; Hong, F.; Yang, P. The Improvement of Spinach Growth by Nano-anatase TiO$_2$ Treatment Is Related to Nitrogen Photoreduction. Biol. Trace Elem. Res. 2007, 119, 77–88. [CrossRef]

26. Su, M.; Liu, H.; Liu, C.; Qu, C.; Zheng, L.; Hong, F. Promotion of nano-anatase TiO$_2$ on the spectral responses and photochemical activities of D1/D2/Cyt b559 complex of spinach. Spectrochim. Acta Part A Mol. Biomol. Spectrosc. 2009, 72, 1112–1116. [CrossRef]

27. Zheng, L.; Hong, F.; Lu, S.; Liu, C. Effect of Nano-TiO$_2$ on Strength of Naturally Aged Seeds and Growth of Spinach. Biol. Trace Elem. Res. 2005, 104, 083–092. [CrossRef]

28. Castiglione, M.R.; Giorgetti, L.; Geri, C.; Cremonini, R. The effects of nano-TiO$_2$ on seed germination, development and mitosis of root tip cells of Vicia narbonensis L. and Zea mays L. J. Nanoparticle Res. 2011, 13, 2443–2449. [CrossRef]

29. Singh, J.; Lee, B.-K. Influence of nano-TiO$_2$ particles on the bioaccumulation of Cd in soybean plants (Glycine max): A possible mechanism for the decline of Cd from the contaminated soil. Environ. Manag. 2016, 170, 88–96. [CrossRef]

30. hogarth, N.; Belcher, M. The contribution of bamboo to household income and rural livelihoods in a poor and mountainous county in Guangxi, China. Int. For. Rev. 2013, 15, 71–81. [CrossRef]

31. Huang, C.; Lin, W.; Lai, C.; Li, X.; Jin, Y.; Yong, Q. Coupling the post-extraction process to remove residual lignin and alter the recalcitrant structures for improving the enzymatic digestibility of acid-pretreated bamboo residues. Bioresour. Technol. 2019, 285, 1355. [CrossRef] [PubMed]

32. State Forestry Administration of China, Statistical Yearbook of Forestry Administration of China: Beijing, China, 2012.

33. Chen, X.; Zhang, X.; Zhang, Y.; Booth, T.; He, X. Changes of carbon stocks in bamboo stands in China during 100 years. For. Ecol. Manag. 2009, 258, 1489–1496. [CrossRef]

34. Zhang, X.; Zhong, T.; Liu, L.; Ouyang, X. Impact of Soil Heavy Metal Pollution on Food Safety in China. PLoS ONE 2015, 10, 5182. [CrossRef] [PubMed]

35. Murashige, T.; Skoog, F. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. Physiol. Plant. 1962, 15, 473–497. [CrossRef]

36. Zhang, X. The Measurement and Mechanism of Lipid Peroxidation and SOD, POD and CAT Activities in Biological System. In Research Methodology of Crop Physiology; Agriculture Press: Beijing, China, 1992.

37. Aebi, H. Catalase in vitro. Methods Enzymol. 1984, 105, 121–126. [CrossRef]

38. Nakano, Y.; Asada, K. Hydrogen Peroxide is Scavenged by Ascorbate-specific Peroxidase in Spinach Chloroplasts. Plant Cell Physiol. 1981, 22, 867–880. [CrossRef]

39. Ellman, G.L. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 1959, 82, 70–77. [CrossRef]

40. Bates, L.S.; Waldren, R.P.; Teare, I.D. Rapid determination of free proline for water-stress studies. Plant Soil 1973, 39, 205–207. [CrossRef]

41. Li, C.; Bai, T.; Ma, F.; Han, M. Hypoxia tolerance and adaptation of anaerobic respiration to hypoxia stress in two Malus species. Sci. Hortic. 2010, 124, 274–279. [CrossRef]

42. Li, K.R.; Feng, C.H. Effects of brassinolide on drought resistance of Xanthoceras sorbifolia seedlings under water stress. Acta Physiol. Plant. 2010, 33, 1293–1300. [CrossRef]

43. Arnon, D.I. Copper Enzymes in Isolated Chloroplasts. Polyphenoloxidase in Beta Vulgaris. Plant Physiol. 1949, 24, 1–15. [CrossRef]

44. Das, K.; Roychoudhury, A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Front. Environ. Sci. 2014, 2, 53. [CrossRef]

45. Lee, S.; Kim, S.; Kim, S.; Lee, I. Assessment of phytotoxicity of ZnO NPs on a medicinal plant, Fagopyrum esculentum. Environ. Sci. Pollut. Res. 2012, 20, 848–854. [CrossRef]

46. You, J.; Chan, Z. ROS Regulation During Abiotic Stress Responses in Crop Plants. Front. Plant Sci. 2015, 6, 1092. [CrossRef]

47. Noctor, G.; Foyer, C.H. ASCORBATE AND GLUTATHIONE: Keeping Active Oxygen Under Control. Annu. Rev. Plant Biol. 1998, 49, 249–279. [CrossRef] [PubMed]

48. Shao, H.B.; Chu, L.Y.; Shao, M.A.; Jaleel, C.A.; Mi, H.M. Higher plant antioxidants and redox signaling under environmental stresses. C. R. Biol. 2008, 331, 433–441. [CrossRef] [PubMed]

49. Trivedi, D.K.; Gill, S.S.; Yadav, S.; Tuteja, N. Genome-wide analysis of glutathione reductase (GR) genes from rice and Arabidopsis. Plant Signal. Behav. 2013, 8, 3021. [CrossRef]

50. Li, J.; Jin, H. Regulation of brassinosteroid signaling. Trends Plant Sci. 2007, 12, 37–41. [CrossRef] [PubMed]

51. Zitka, O.; Skalickova, S.; Gumulec, J.; Masarik, M.; Adam, V.; Hubalek, J.; Trnkova, L.; Kruseova, J.; Eckschläger, T.; Kizek, R. Redox status expressed as GSH:GSSG ratio as a marker for oxidative stress in paediatric tumour patients. Oncol. Lett. 2012, 4, 1247–1253. [CrossRef]
52. Movafeghi, A.; Khataee, A.; Abedi, M.; Tarrahi, R.; Dadpour, M.; Vafaei, F.; Abedi, M. Effects of TiO$_2$ nanoparticles on the aquatic plant Spirodela polyrrhiza: Evaluation of growth parameters, pigment contents and antioxidant enzyme activities. *J. Environ. Sci.* 2018, 64, 130–138. [CrossRef]

53. Song, G.; Gao, Y.; Wu, H.; Hou, W.; Zhang, C.; Ma, H. Physiological effect of anatase TiO$_2$ nanoparticles on Lemma minor. *Environ. Toxicol. Chem.* 2012, 31, 2147–2152. [CrossRef] [PubMed]

54. Spengler, A.; Wanninger, L.; Pfugmacher, S. Oxidative stress mediated toxicity of TiO$_2$ nanoparticles after a concentration and time dependent exposure of the aquatic macrophyte Hydrilla verticillata. *Aquat. Toxicol.* 2017, 190, 32–39. [CrossRef]

55. Hasan, K.; Liu, C.; Wang, F.; Ahammed, G.J.; Zhou, J.; Xu, M.X.; Yu, J.Q.; Xia, X.J. Glutathione-mediated regulation of nitric oxide, S-nitrosothiol and redox homeostasis confers cadmium tolerance by inducing transcription factors and stress response genes in tomato. *Chemosphere* 2016, 161, 536–545. [CrossRef] [PubMed]

56. Anjum, N.A.; Ahmad, I.; Mohmood, I.; Fachecco, M.; Duarte, A.C.; Pereira, E.; Umar, S.; Ahmad, A.; Khan, N.A.; Iqbal, M.; et al. Modulation of glutathione and its related enzymes in plants’ responses to toxic metals and metalloids—A review. *Environ. Exp. Bot.* 2011, 75, 307–324. [CrossRef]

57. Anjum, N.A.; Gill, S.S.; Gill, R.; Hasanuzzaman, M.; Duarte, A.C.; Pereira, E.; Ahmad, I.; Tuteja, R.; Tuteja, N. Metal/metalloid stress tolerance in plants: Role of ascorbate, its redox couple, and associated enzymes. *Protoplasma* 2014, 251, 1265–1283. [CrossRef]

58. Silva, S.; De Oliveira, J.M.P.F.; Dias, M.C.; Silva, A.M.; Santos, C. Antioxidant mechanisms to counteract TiO$_2$-nanoparticles toxicity in wheat leaves and roots are organ dependent. *J. Hazard. Mater.* 2019, 380, 889. [CrossRef] [PubMed]

59. LaRue, C.; Michel, C.H.; Sobanska, S.; Trcera, N.; SorieuL, S.; Céclillon, L.; Ouerdane, L.; Legros, S.; Sarret, G. Fate of pristine TiO$_2$ nanoparticles and aged paint-containing TiO$_2$-nanoparticles in lettuce crop after foliar exposure. *J. Hazard. Mater.* 2014, 273, 17–26. [CrossRef]

60. Noctor, G.; Mhamdi, A.; Chaouch, S.; Han, Y.; Neukermans, J.; García, M.B.; Queval, G.; Foyer, C.H. Glutathione in plants: An integrated overview. *Plant Cell Environ.* 2011, 35, 454–484. [CrossRef] [PubMed]

61. Kishor, P.B.K.; Sreenivasulu, N. Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue? *Plant Cell Environ.* 2013, 37, 300–311. [CrossRef]

62. Ashraf, M.; Foolad, M. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* 2007, 59, 206–216. [CrossRef]

63. Chandrakar, V.; Yadu, B.; Meena, R.K.; Dubey, A.; Keshavkant, S. Arsenic-induced genotoxic responses and their amelioration by diphenylethylenone, 24-epibrassinolide and proline in Glycine max L. *Plant Physiol. Biochem.* 2017, 112, 74–86. [CrossRef]

64. Hayat, S.; Hayat, Q.; Alyemeni, M.N.; Wani, A.S.; Pichtel, J.; Ahmad, A. Role of proline under changing environments. *Plant Signal. Behav.* 2012, 7, 1456–1466. [CrossRef]

65. Latef, A.A.A.H.; Srivastava, A.K.; Sadek, E.M.S.A.; Kordrostami, M.; Tran, L.S.P. Titanium Dioxide Nanoparticles Improve Growth and Enhance Tolerance of Broad Bean Plants under Saline Soil Conditions. *Land Degrad. Dev.* 2018, 29, 1065–1073. [CrossRef]

66. Sharma, P.; Jha, A.B.; Dubey, R.S.; Pessarakli, M. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *J. Bot.* 2012, 1, 26. [CrossRef]

67. Sharma, P.; Jha, A.B.; Dubey, R.S. *Handbook of Plant and Crop Stress*; CRC Press: Boca Raton, FL, USA, 2016; pp. 109–158.

68. Mohammadi, R.; Amiri, M.R.; Abbasi, A. Effect of TiO$_2$ Nanoparticles on Chickpea Response to Cold Stress. *Biol. Trace Elem. Res.* 2013, 152, 403–410. [CrossRef]

69. Karami, A.; Sepehri, A. Nano titanium dioxide and nitric oxide alleviate salt induced changes in seedling growth, physiological and photosynthesis attributes of barley. *Zemdirb. Agric.* 2018, 105, 123–132. [CrossRef]

70. Silva, S.; Craveiro, S.C.; Oliveira, H.; Calado, A.J.; Pinto, R.J.; Silva, A.M.; Santos, C. Wheat chronic exposure to TiO$_2$-nanoparticles: Cyto- and genotoxic approach. *Plant Physiol. Biochem.* 2017, 121, 89–98. [CrossRef]

71. Moore, M.N. Environmental risk management—The state of the art. *Environ. Int.* 2006, 32, 967–976. [CrossRef]

72. Gao, F.; Liu, C.; Qu, C.; Zheng, L.; Yang, F.; Su, M.; Hong, F. Was improvement of spinach growth by nano-TiO$_2$ treatment related to the changes of Rubisco activase? *BioMetals* 2007, 21, 211–217. [CrossRef] [PubMed]

73. Hong, F.; Yang, F.; Liu, C.; Gao, Q.; Wan, Z.; Gu, F.; Wu, C.; Ma, Z.; Zhou, J.; Yang, P. Influences of Nano-TiO$_2$ on the Chloroplast Aging of Spinach Under Light. *Plant Biol.* 2013, 104, 249–260. [CrossRef]

74. Shabbir, A.; Khan, M.; Ahmad, B.; Sadiq, Y.; Jaleel, H.; Uddin, M. Efficacy of TiO$_2$ nanoparticles in enhancing the photosynthesis, essential oil and khusimol biosynthesis in Vetiveria zizanioides L. Nash. *Photosynthetica* 2019, 57, 599–606. [CrossRef]

75. Gururani, M.A.; Venkatesh, J.; Tran, L.S.P. Regulation of Photosynthesis during Abiotic Stress-Induced Photoinhibition. *Mol. Plant* 2015, 8, 1304–1320. [CrossRef]

76. Morteza, E.; Moaveni, P.; Farahani, H.A.; Kiyani, M. Study of photosynthetic pigments changes of maize (Zea mays L.) under nano TiO$_2$ spraying at various growth stages. *SpringerPlus* 2013, 2, 247. [CrossRef]

77. Jiang, F.; Shen, Y.; Ma, C.; Zhang, X.; Cao, W.; Rui, Y. Effects of TiO$_2$ nanoparticles on wheat (Triticum aestivum L.) seedlings cultivated under super-elevated and normal CO$_2$ conditions. *PLoS ONE* 2017, 12, 8088. [CrossRef] [PubMed]

78. Yang, F.; Hong, F.; You, W.; Liu, C.; Gao, F.; Wu, C.; Yang, P. Influences of Nano-anatase TiO$_2$ on the Nitrogen Metabolism of Growing Spinach. *Biol. Trace Elem. Res.* 2006, 110, 179–190. [CrossRef]

79. Mishra, V.; Mishra, R.K.; Dikshit, A.; Pandey, A.C. Interactions of nanoparticles with plants: An emerging prospective in the agriculture industry. In *Emerging Technologies and Management of Crop Stress Tolerance: Biological Techniques*, 1st ed.; Ahmad, P., Rasool, S., Eds.; Academic Press: Cambridge, MA, USA, 2014; pp. 159–180.