

Biological Cadmium Detoxification in Rice Using the Fungus *Trametes Pubescens*: A Promising and Feasible Approach

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Abstract

Background: Cadmium is a widely dispersed metal element that may be released to the environment with accelerated anthropogenic activities. Rice with high cadmium content is a serious threat to food safety and human health.

Results: A heavy metal-resistant white rot fungus *Trametes pubescens* MB 89 with remarkable ability to prevent rice from cadmium toxification has been investigated in this study. We have assessed the biosorption of this ectomycorrhizal fungus with high concentrations of Cd^{2+} . Results indicate that the maximum removal rate of Cd^{2+} from aqueous solution (10 mg/L) by *T. pubescens* MB 89 was 53.13%. A biofilm was formed on the cell surface of mycelium with Cd (II) been reduced to Cd (0) in the form of nanoscale particles in aqueous solution. It was found that the functional groups of $-\text{OH}$, $-\text{NH}$, C-H , C=O , C-O , S=O on the surface of *T. pubescens* MB 89 participated in the Cd^{2+} detoxification process. The effect of rice plant colonization with *T. pubescens* MB 89 was investigated with the pot experiments showing that it could significantly promoted rice seedlings growth and reduced cadmium accumulation on rice tissues. Moreover, the fungal colonization could decrease the cadmium transport to the rice roots, ameliorate cadmium toxicity and ensure the normal absorption and transportation of nutrients by the roots.

Conclusion: This study showed a promising potential for *T. pubescens* MB 89 to prevent cadmium toxification for the rice grains in paddy fields. To further investigate the mechanism of *T. pubescens* to protect and promote rice growth, our next research focus will be on the beneficial interactions of ectomycorrhizal fungi and the rice root system with the aid of the root-fungal interactome analysis.

Introduction

The rapid technological development and urbanization coupled with population growth is posing significant impacts on the environment, ecosystems and human society worldwide. As a result, the farmland pollution is concerned as one of the most severe global issues that has prompted all countries in the world to pay more attention on the remediation of agricultural lands for the sustainable development of economy (Wei et al., 2020; Xie et al., 2019; Zhang et al., 2020)

Cadmium is a widely dispersed metal element that may be released to the environment with accelerated anthropogenic activities. Cadmium is active in the migration between the soil-plant rhizosphere and is toxic to animals, plants and humans (Martelli et al., 2006b; Wang et al., 2020a). With the increase of modern industrial and agricultural production, as well as the use of pesticides and fertilizers, it has caused an elevated cadmium release to the environment (Taiwo et al., 2016). It is estimated that about 22 thousand metric tons of cadmium enters the farmland in the world each year (Xu et al., 2014). Moreover, Cadmium pollution is an irreversible process which can barely be remediated by soil microbiota. Once absorbed, cadmium may remain toxic in ecosystems or human bodies for more than 20 years (Martelli et al., 2006a). On the other hand, cadmium is a highly mobile element, which is easy to be absorbed and

accumulated by plants in cropland (Chen et al., 2018b; Sarwar et al., 2017). As a long term impact, cadmium may not only inhibited the yield and quality of crops seriously, but also enter human bodies through the food chain to accumulate and cause damage to human health (Liu et al., 2013; Shan et al., 2020). The transfer route of heavy metals into human beings is from soil through crops to food and then to human body (Aziz et al., 2015). Once cadmium accumulates in human blood, it will gradually interfere with renal metabolism and bone formation *in vivo* (Chen et al., 2018a). Even at a low level, long-term presence of cadmium poses a serious threat to human health (Mishra et al., 2018; Yaghoubian et al., 2016).

Rice is one of the most important crops as the main food source for more than 50% of the global population. The quality of rice is directly related to people's health (Aziz et al., 2015). However, in recent years, the occurrence of high cadmium rice, or the rice with excessive cadmium content, is concerned to the public. Continuous consumption of food containing cadmium above 0.2–0.3 mg/Kg may cause itai-itai disease with pains in the waist, hands, feet and other joints. After a few years, the patients may experience neuralgia, bone pains, difficult moving, and even breathing pains (Inaba et al., 2005; Suwazono et al., 2019).

It has been found that some soil microorganisms have developed survival strategies in high Cd-contaminated environment and be able to reduce Cd bioavailability (Bai et al., 2008; Limcharoensuk et al., 2015). In analogy to the behavior of soil microorganisms, approaches have been used to suppress Cd accumulation in the rice grains in paddy fields (Lin et al., 2016). There are several approaches of microbial remediation for cadmium pollution, including biosorption, biomineralization, biotransformation and bioaccumulation (Ayangbenro and Babalola, 2017). Many researchers have explored the rice contamination through the rhizosphere within a Cd-rich environment (Patten and Glick, 2002). Exogenous treatment with indole-3-acetic acid is seen to alleviate Cd accumulation in shoots and roots of wheat seedlings (Agami and Mohamed, 2013). A cadmium resistant *Ochrobactrum sp.* capable of producing siderophore, ACC deaminase and catalase, is found to reduce the metal toxicity to rice (Pandey et al., 2013). It is well known that fungi are able to tolerate and detoxify heavy metal contaminated effluents (Jacob et al., 2018). However, due to the high toxicity of cadmium, so far few fungi have been used as biological adsorbents to prevent rice from cadmium contamination.

T. pubescens MB 89 is a white rot fungus that can produce laccase in the presence of copper ion (Galhaup and Haltrich, 2001; Tian et al., 2018). While it has strong resistance to heavy metals, *T. pubescens* has been reported for its use in wastewater treatment (Strong and Burgess, 2007; Strong and Burgess, 2008). There are no studies so far on its removal of cadmium from rice. In this study, *T. pubescens* MB 89 was studied for its resistance against cadmium toxicity and promotion of rice growth with elimination of cadmium. Our specific objectives were: (i) To evaluate the effects of cadmium on the growth of *T. pubescens* MB 89 and the its Cd adsorption; (ii) To explore Cd biomineralization by *T. pubescens* MB 89 using Scanning Electron Microscope (SEM) and Fourier Transform Infrared Spectroscopy (FTIR); (iii) Finally, to investigate *T. pubescens* MB 89 colonization on the rice growth and cadmium removal from paddy tissue by pot hydroponic experiments.

Results And Discussion

Cd tolerance of *T. pubescens* MB 89 strain

Growth inhibition is a common response of plants to heavy metal stress. Cadmium is one of the most toxic heavy metals that can affect soil quality and reduce plant productivity. Furthermore, the high cadmium rice is a serious threat to food safety and human health. In this work, we used a cadmium-tolerant *T. pubescens* to investigate its resistance against cadmium stress. The radius growth diameter of the strain in PDA plate were measured every 24 h with different concentration gradient of Cd^{2+} , as shown in Fig. 1. It can be seen that *T. pubescens* MB 89 grew well at the Cd^{2+} concentration of 10 mg/L. With the increase of cadmium concentration, the growth of *T. pubescens* was gradually inhibited. When Cd^{2+} concentration reached 200 mg/L there was no hypha observed. Cadmium tolerance assay showed that strain *T. pubescens* MB 89 has remarkable tolerance to cadmium. According to the literature, under solid cultivation, the MICs for Cd^{2+} of *Aspergillus sp. 2* and *Penicillium sp.* isolated from metal-contaminated soils were 35.59 and 44.48 mM, respectively (Zafar et al., 2007). The MIC of *Rhizopus sp.* for Cd^{2+} was 17.8 mM (IQBAL AHMAD, 2005). Figure 1.

Cadmium removal by *T. pubescens* mycelium

As shown in Fig. 2, the cadmium removal by *T. pubescens* MB 89 from the aqueous solution was investigated. It is observed that with the initial Cd^{2+} concentration of 100 mg/L, the Cd adsorption of *T. pubescens* living mycelium was 6.565 mg/g which was the highest in this experiment. The maximum removal rate of *T. pubescens* MB 89 was 53.13% when the initial cadmium concentration was 10 mg/L. while a literature reported the removal efficiency of *A. aculeatus* reached the maximum (46.8%) at 10 mg/L Cd^{2+} concentrations (Xie et al., 2019). Since this process was affected by the mycelium's cell surface substances and metabolism, the cadmium removal by mycelia in aqueous solution depends on complex factors (Arivalagan et al., 2014). From Fig. 2 it can be seen that biosorption capacity increased with increased initial concentration of Cd^{2+} before it was saturated. This result was in agreement with the literature in which Cd^{2+} biosorption capacity of living fungus *Pseudomonas sp. 375* was studied (Xu et al., 2020). Figure 2.

SEM analysis

Jacob et al. (Jacob et al., 2017) have reported the alteration of cell wall morphology due to heavy metal stress. Figure 3 Shows the SEM micrograph of Cd treated and untreated (control) *T. pubescens* MB 89 cells. It confirms that heavy metals were able to inhibit various physiological processes such as cell membrane distribution (Karthik et al., 2016; Khan et al., 2009; Yuan et al., 2015), inhibited cell division, depressed enzyme activity, and caused denaturation of protein (Khan et al., 2009; Wyszowska et al., 2014). As shown in Fig. 3b, a large number of mycelia were deformed and became fragmented when the

cadmium concentration in the medium was too high for the fungus to bear. Even in the shaking bottle, the mycelia originally growing together had fragments falling down. Nevertheless, dense nanoscale particles on the cell surface of mycelium were observed in the low Cd experimental groups (Fig. 3d), while these particles were not seen in the control group (Fig. 3a). This means the dense particles were Cd (0) sediments formed after *T. pubescens* had adsorbed and reduced Cd (II). The phenomenon is similar to the work with *Pseudomonas chengduensis* strain MBR (Wang et al., 2020b). The fungal detoxification of heavy metal contaminated environment includes valence transformation, intra and extracellular precipitations as well as active uptake (Thatoi et al., 2014). Typically, the high content of carboxyl groups in the mannuronic and guluronic acids of the cell wall polysaccharides and the protosufficiency enhance heavy metal biosorption (Raja et al., 2016). Figure 3.

FTIR analysis

The FTIR analysis was implemented to verify the metal ion interacting with the functional groups existing on the fungal surface in the wavelength of 500–4000 cm^{-1} . The FTIR spectra of *T. pubescens* MB 89 exposed to Cd showed varying asymmetrical stretching bands and peaks in Fig. 4. The major variations in peaks are shown in Table 1. Among them, the stretching vibration peaks of amino and hydroxyl groups were found to shift from 3487.32 cm^{-1} (control group) to 3419.93 cm^{-1} (5 mg/L Cd), 3405.40 cm^{-1} (10 mg/L Cd), 3390.31 cm^{-1} (50 mg/L Cd) and 3402.99 cm^{-1} (100 mg/L Cd), indicating that these hydroxyl groups and amino groups from polysaccharide, fatty acid and protein components were participated in the adsorption process (Zhou et al., 2016). In addition, there is a C-H stretching vibration peak near 2928 cm^{-1} , and the slight shift of the spectrum after adsorption indicate that C-H of methyl groups was participated in the cadmium adsorption process (Khan et al., 2018). The carbonyl stretching vibration of amide and –NH distortion bands was observed at 1651 cm^{-1} (Kuyucak and Volesky, 1989). The spectrum shows the low-intensity vibration deviation of band 1077 cm^{-1} , the peak that shifted from 1084 cm^{-1} to 1075 cm^{-1} could be attributed to the C-O stretching of carboxyl groups and S = O groups (Loukidou et al., 2003). To sum up, it can be inferred that the functional groups of –OH, –NH, C-H, C = O, C-O, S = O existing on the surface of *T. pubescens* MB 89 might participate in the Cd^{2+} biosorption process. Table 1.

Table 1
The shift of absorption peak band in FTIR spectra of *T. pubescenceat* different cadmium concentrations

IR peaks	Wavenumber(cm-1)					Association
	control	5 mg/L	10 mg/L	50 mg/L	100 mg/L	
1	3487.32	3419.93	3405.4	3390.31	3402.99	bonded-OH, -NH Stretching
2	2928.13	2928.29	2928.96	2928.30	2928.74	C-H Stretching
3	1651.29	1651.78	1653.14	1651.94	1644.45	C = O Stretch of COOH
4	1403.71	1401.84	1402.42	1404.51	1413.96	C-H Stretching
5	1077.98	1079.33	1079.75	1077.86	1076.57	C-O and S = O Stretching

1 Effect of *T. pubescens* colonization on growth of rice plants under Cd stress

Rice seedlings were grown in a plant tissue culture chamber exposed to a specific temperature, humidity, and light conditions. Cd stress experiments were performed by adding CdCl₂ to a final concentration of 10 mg/L. Under Cd stress, rice seedling growth was significantly inhibited, which was characterized by short plant height and yellow withered leaves (Fig. 5a). However, there was remarkable difference observed between the growth of rice seedlings colonized with *T. pubescens* and CONT group without Cd stress. Further measurement results (Fig. 5b, d, e) indicated that the addition of Cd²⁺ had inhibited growth of rice seedlings with both the height of shoots and the length/diameter of root decreased significantly. It was found that the shoot height was almost 38.46% less in Cd²⁺ treated seedlings than non-treated ones. On the other hand, the root length was almost 52.5% less in Cd²⁺ treated seedlings than non-treated seedlings. Furthermore, the root diameter was almost 49.2% less in Cd²⁺ treated seedlings than non-treated seedlings. Therefore, Cd²⁺ had an apparent toxic effect on rice seedlings, especially on the roots, this results were in close agreement with the work in literature (Wang et al., 2019). Figure 5.

In this study, we were surprised to find that the colonization of *T. pubescens* had remarkably reduced the cadmium toxicity and relieved its inhibition on rice seedlings. Moreover, it showed to promote the growth of rice seedlings, showing healthier growth state and larger roots. Therefore, *T. pubescens* rhizosphere was seen to possess the ability to counteract Cd-stress.

2 *T. pubescens* colonization on plant tissues cadmium accumulation against Cd toxicity

Regardless of the presence or absence of microorganism, roots usually accumulated more Cd than that in culms, leaves and grains (Shan et al., 2020). After 15 days of growth in Cd-containing (10 mg/mL) aqueous culture medium, the Cd accumulation in roots and shoots of the rice plants are showed in Fig. 6. For seedlings colonized by *T. pubescens*, Cd concentrations in roots and shoots were significantly reduced by 53.54% and 86.38%, respectively. These results clearly show that *T. pubescens* was able to suppress the accumulation of Cd in rice. It thus has positive potential on heavy metal removal for more sustainable agriculture. Our experimental outputs were consistent with previous studies by other workers (Lin et al., 2016; Zhou et al., 2016), in which some other Cd-tolerant microorganisms were used. Figure 6.

3 *T. pubescens* colonization on rice roots against Cd toxicity

Evidences has suggested that active growth of the roots accelerated the absorption of nutrients and thereby facilitated shoot growth(Cai et al., 2020). As shown in Fig. 7 (with root cross section slices g, h, and i), cadmium contents in rice plants have caused serious damage to the cell morphology of roots, and further resulted in cell deformation and senescence. The *T. pubescens* colonization has protected root cells from disrupture. As a result of cadmium adsorption by *T. pubescens*, the residual cadmium concentration in the medium was significantly reduced, which alleviated the toxicity of cadmium to roots. It is seen from the vertical section of rice roots (Fig. 7a, b, c, d, e, f) that the CONT(Cd) with cadmium showed retarded plant development with cell degeneration of the root tips, root thinning. Obviously, cadmium in excess in the soil interfered with the uptake and transport of mineral nutrients by roots, resulting in nutrient deficiency of plants. Furthermore, cadmium ion might degrade root tip cells, switched down water absorption and transport system, it thus resulted in reduced nutrient supply. In comparison, development of the root tips for the rice plants in (Tp(Cd)) group were healthy and similar to that for CONT group. As can be seen from the comparison between Fig. 7b and c, *T. pubescens* colonization on rice plants could significantly prevent the rice roots from the damage by environmental cadmium, protect the rice root tip cells, and enable absorption and transportation of nutrients from the rhizosphere micro-environment. The results suggested that *T. pubescens* has played an important roles in protecting rice plants from Cd-induced damages. Figure 7.

Conclusions

A heavy metal-resistant white rot *T. pubescens* MB 89 strain was studied for its remarkable tolerance to cadmium. Investigation of *T. pubescens* MB 89 indicates that the maximum Cd removal rate from aqueous solution was 53.13% when the initial cadmium concentration was 10 mg/L. SEM analysis of *T. pubescens* MB 89 confirmed that when the cadmium concentration in the medium was too high for the fungus to bear, the rice plant physiological status and cell metabolism were changed, and a large number of mycelia were deformed and became fragmented. However, dense nanoscale particles on the cell surface of mycelium were observed in the low Cd experimental groups. Cd (0) sediments were formed after *T. pubescens* has worked to reduce the concentration of Cd (II) in aqueous solution. FTIR spectral

analysis demonstrated that the functional groups of –OH, –NH, C-H, C = O, C-O, S = O existing on the surface of *T. pubescens* MB 89 might participate in the Cd²⁺ biosorption process. The results from pot experiments showed that *T. pubescens* MB 89 colonization on rice plants has significantly promoted growth of rice seedlings and reduced cadmium accumulation on rice tissues. Moreover, *T. pubescens* MB 89 colonization could significantly decrease the cadmium transport to the rice roots, counteract cadmium toxicity and ensure the normal absorption and transportation of nutrients by the roots. This study shows a promising potential for *T. pubescens* to remove cadmium from the rice grains in paddy fields.

It is the first time as we know that white rot fungus *T. pubescens* was used to prevent rice roots from cadmium toxification. However, there are several issues that remain to be solved. First, from the section results (Fig. 7), we can postulate that *T. pubescens* have a protective effect on plant roots by secretion of cadmium degrading enzymes; Second, from the observation in this study it is obvious that the presence of *T. pubescens* in the medium would enhance the root and shoot development of the rice, it could be supposed that there existed some phytohormones that are able to stimulate rice growth. To further investigate the mechanism of *T. pubescens* to protect and promote rice growth, our next research focus will be on the beneficial interactions of ectomycorrhizal fungi and the rice root system with the aid of the root-fungal interactome analysis.

Materials And Methods

Fungal strains and cultivation

T. pubescens MB 89 (The Netherlands, CBS 696.94) was obtained from Dr. Kheirghadam Enayatzamir (Shahid Chamran University, Ahvaz, Iran). The spores of *T. pubescens* were inoculated in a sterilized liquid medium (10 g glucose, 20 g peptone from casein, 0.9 g (NH₄)₂SO₄, 2 g KH₂PO₄, 0.5 g MgSO₄·7H₂O, 0.1 g CaCl₂·2H₂O, 0.5 g KCl, 0.5 g thiamine per liter of medium, and 20% citrate phosphate buffer solution, pH 4.5) in 28 °C incubator for 15 days. In order to stimulate laccase production, 0.5 mM Cu²⁺ was supplemented on third day of cultivation. The fungus was maintained on potato dextrose agar plate at 4 °C.

Cd resistance

The Cd resistance of *T. pubescence* was investigated by monitoring its radial growth on PDA medium (1/4-Strength PDA) containing different concentrations of Cd²⁺ (0, 10, 25, 50, 100, 200 ppm). A plug of fungal inoculum was placed on medium and incubated at 28 °C for 6 days. Radial growth of *T. pubescence* was measured daily and the inhibition percentage of fungal growth was evaluated.

Cd Adsorption Capacity

Cd adsorption test was carried out in 250 mL Erlenmeyer flasks filled with 100 mL liquid medium with different concentration of Cd²⁺ (0, 5, 10, 50, 100 ppm) addition on 8th day of cultivation. The culture flasks were placed in an incubator at a constant temperature of 28 °C. After adding cadmium, flasks were moved from the incubator to a shaker to culture at a constant rotation rate of 130 rpm. Cd adsorption Test was conducted for another 7 days. The *T. pubescence* biomass was collected and freeze-dried for further study. After adsorption, the residual broth was examined for the cadmium ion concentration in the solution. The Cd ions absorbed by the fungus were calculated via the following equation (Xu et al., 2012):

$$q_e = \frac{V \times (C_i - C_e)}{W} \quad (1)$$

$$\text{Adsorption rate}(\%) = \frac{(C_i - C_e)}{C_i} \times 100\% \quad (2)$$

where q_e is the Cd uptake in mg Cd²⁺ kg⁻¹ biomass; V is the value of the metal-containing solution in mL; C_i and C_e are the initial and equilibrium concentrations of Cd²⁺ in the solution, respectively, in mg L⁻¹; and W is the weight of dry mycelia in g.

FTIR and SEM analysis

The functional groups of fungal cell surface area before and after Cd²⁺ treatment were analyzed by infrared spectroscopy (FTIR, Perkin Elmer Frontier, Perkin Elmer Inc., USA) after freeze-drying. The KBr matrix (Sigma) and the scanning wavelength in the range of 500–4000 cm⁻¹ were applied in this process. The scanning electron microscope (SEM, Hitachi S-4800Hitachi, Ltd, Chiyoda-ku, Japan) was used to analyze the morphological structure and to evaluate the cadmium adsorption.

Construction of fungus-rice co-culture system for prevention of cadmium accumulation in rice

The fungus-rice co-culture system was consisted of the three parts: *T. pubescens* seed culture, plant samples preparation and co-growth in the high concentration of cadmium environment.

The rice seeds were obtained from Jiangmen city in Guangdong province, china. Seeds were sterilized with 5% H₂O₂ and germinated on filter paper at 25 °C in the dark for 3–4 days. After germination, uniform length of plantlets were transferred into small trays with fixed plastic cups (4 cm diameter and 5 cm height, twelve plants per cup) and grown hydroponically in Hewitt nutrient medium (Liu et al., 2004) for 7 d for acclimatization. At this stage, the seedlings were placed in a light incubator (16 h light /8 h dark) at a constant temperature of 26°C.

T. pubescens was grown in 1000 ml Erlenmeyer flask containing 100 ml of above mentioned culture medium. They were statically incubated at 28 °C, supplemented with 0.5 mM Cu²⁺ to stimulate laccase production on third day of cultivation. After 10 days cultivation the spores and liquid solution containing laccase were used as bioremediation agents.

Subsequently, 27 cups of rice were divided into three groups, namely, the control (CONT), Cd treatment only (CONT(Cd)) and Cd plus *T. pubescence* (Tp(Cd)). For the Cd + *T. pubescence* and Cd groups, the concentration of Cd²⁺ were 10 ppm. Moreover, the prepared bioremediation agents were added into the Tp(Cd) group. The plants were grown in a tissue culture room at 25 ± 2 °C with 14 h light/10 h dark and 60% relative humidity for 15 days.

Morphological observation of rice plants

After 15 days of cultivation, the plants were harvested and the morphological observation of rice in different groups was carried out. The plants were washed with sterilized distilled water, then the root and shoot length of the plants and root-collar diameter were measured by a metric scale. In addition, plant development in terms of the number of leaves and branches was recorded. Paraffin section was used to observe the cell morphology of roots. Dry and fresh weight of the aerial part and roots were determined. At the end of the experiment, the plants were frozen in fridge and stored at - 80 °C for further analysis.

Cadmium accumulation in rice tissue quantification

Cadmium accumulation in rice root and shoot was estimated as follows: After completion of 15 d, harvested plants were washed thoroughly with demineralized water, blotted, and oven dried (80 °C) for 1 d. 0.2 g dried powder of root and shoot was digested in 3 ml HNO₃ at 120 °C for 6 h (Awasthi et al., 2018). After digestion, the volume was constant to 50 ml by Milli-Q water. Cd accumulation in cellular shoot and root was quantified by atomic absorption spectrometry, respectively.

Statistical analysis

All the experiments were conducted at three replications and the means of replications are presented along with standard deviation calculated by the software SPSS 17.0.

Declarations

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Availability of data and materials

Not applicable.

Authors' contributions

PCF, NE and JL conceived and designed the experiment. JL, WL and XYL conducted the experiments, performed data analysis and wrote the manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

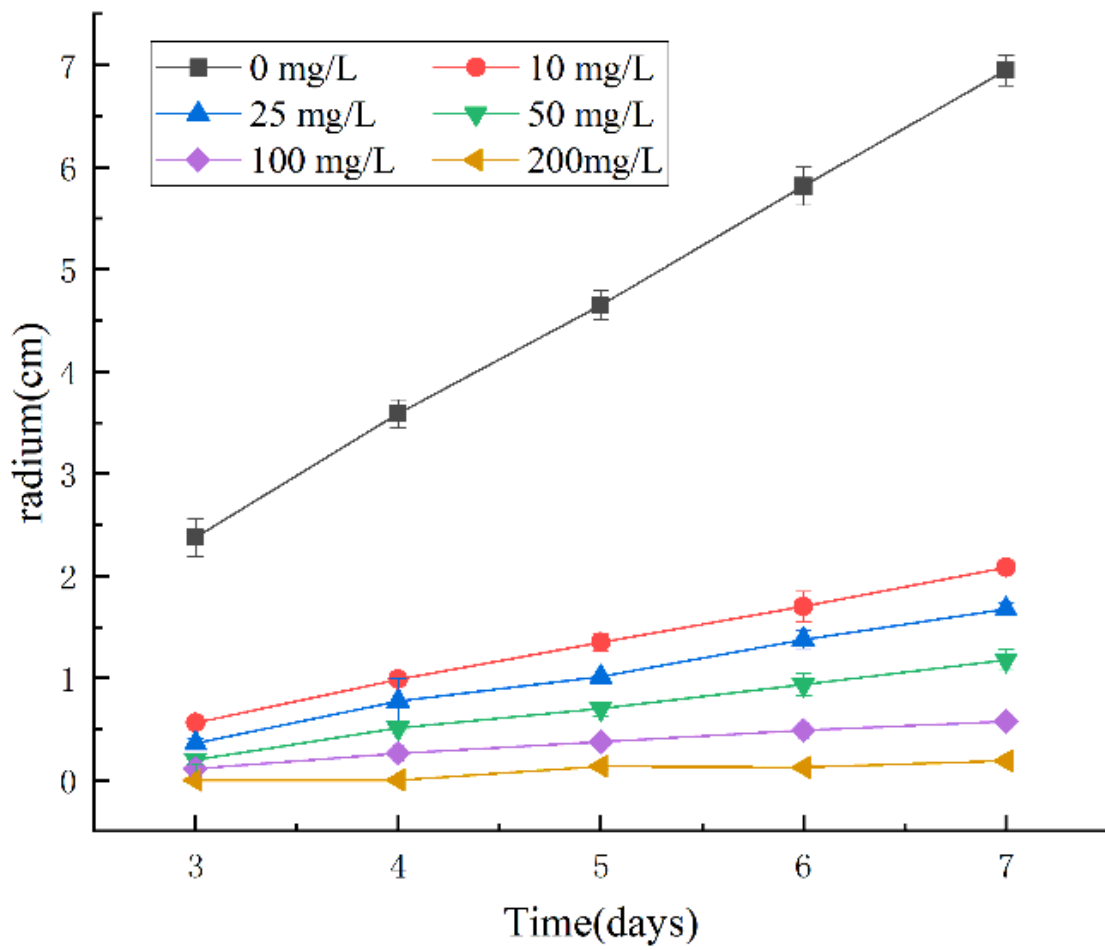


Figure 1

Growth inhibition of *T. pubescens* MB 89 with varying Cd^{2+} concentrations.

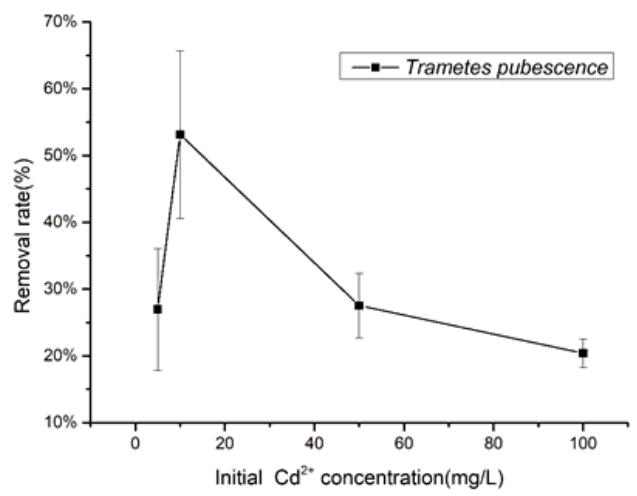
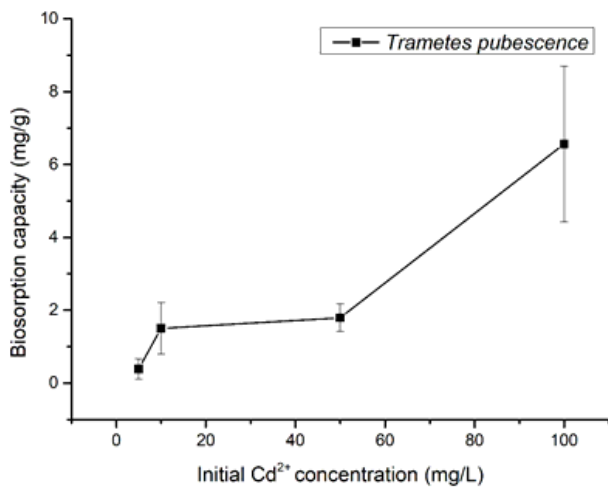


Figure 2

Removal rate of cadmium by *T. pubescens* MB 89 with varying Cd²⁺ concentrations.

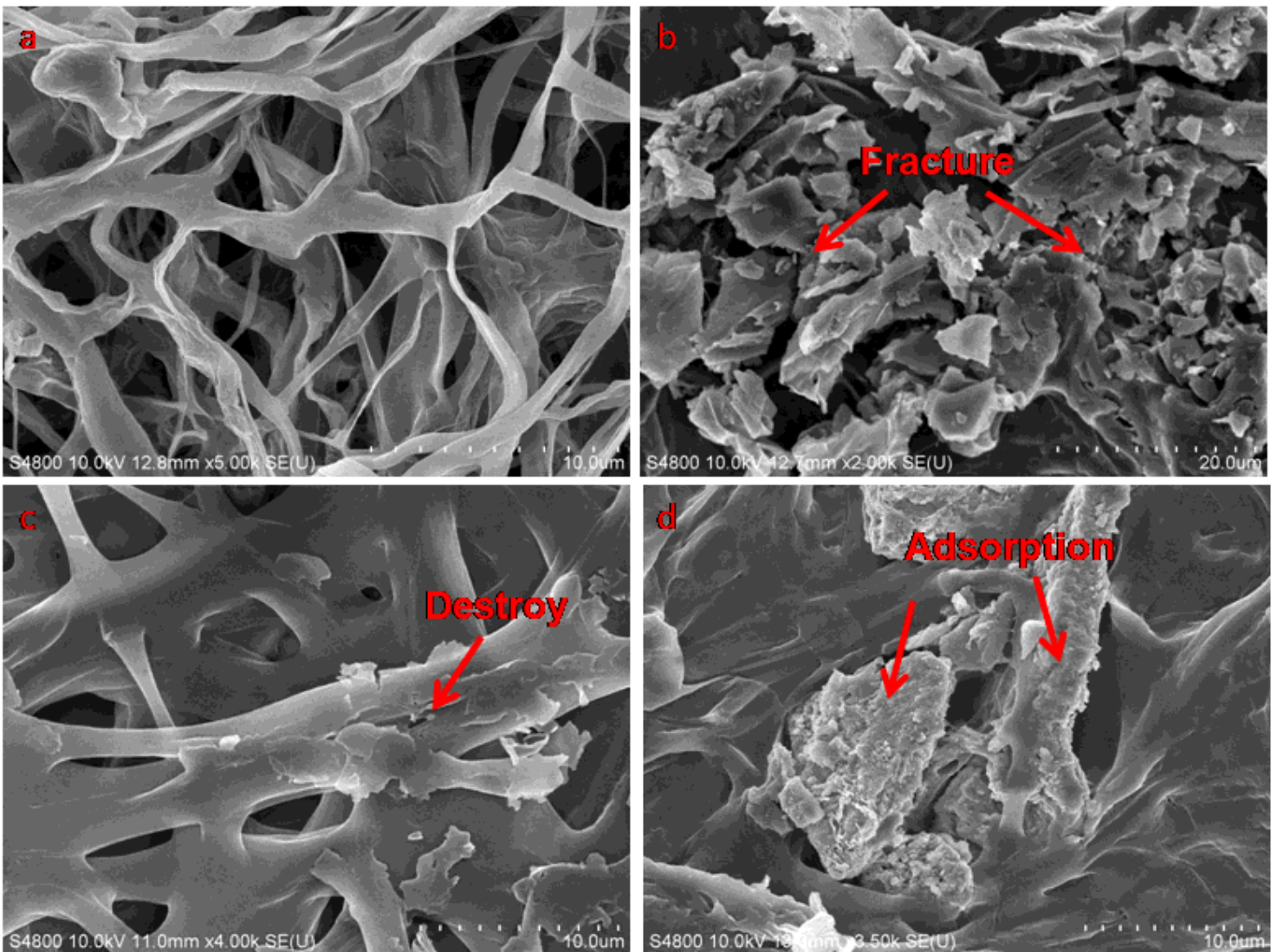


Figure 3

SEM micrograph of *T. pubescens* MB 89 biomass before and after high/low concentration of Cd²⁺ treatment. a: without Cd²⁺ treatment (CONT); b: treated with high concentration of Cd²⁺; c and d: treated with low concentration of Cd²⁺.

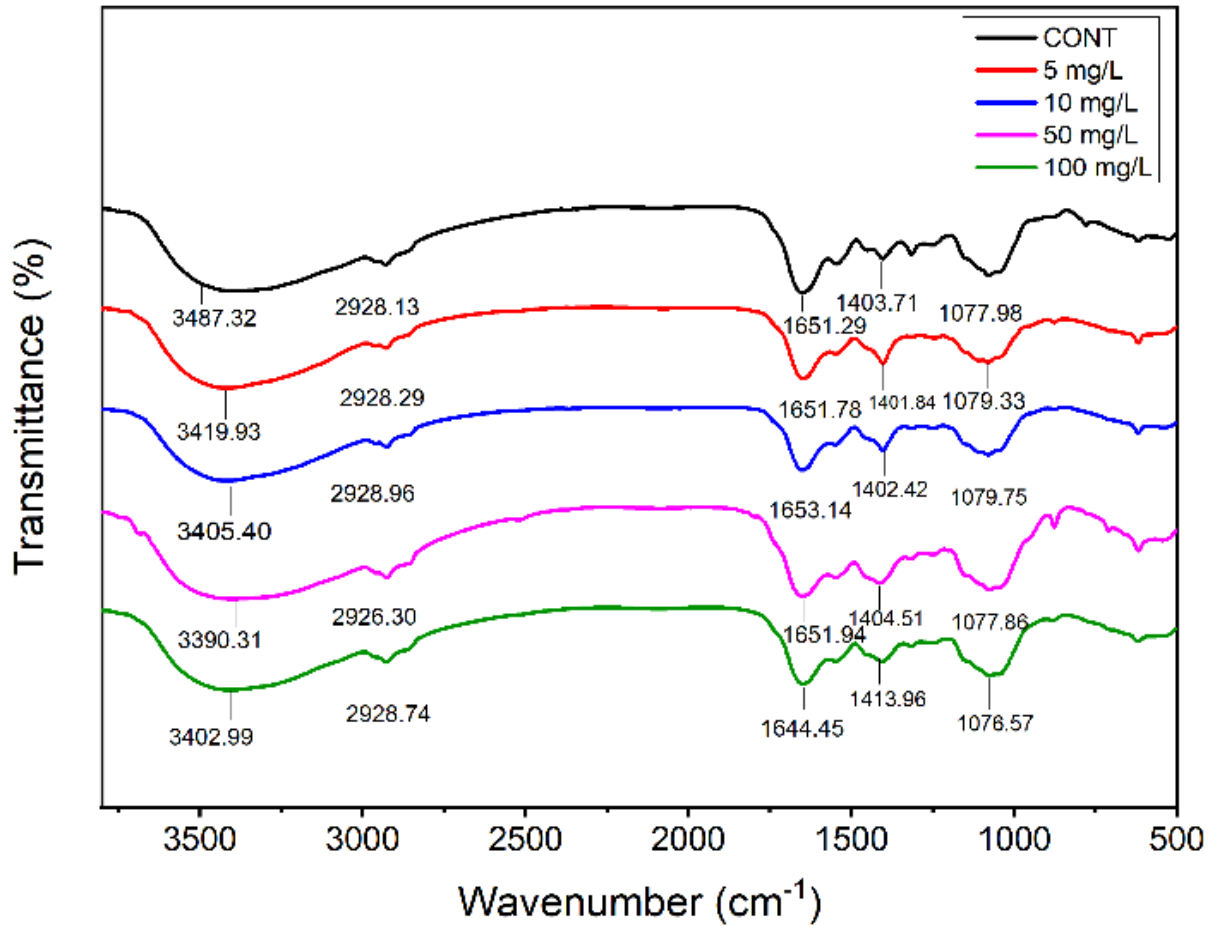


Figure 4

FTIR spectra of *T. pubescens* dry biomass with varying Cd²⁺ concentrations Enhanced growth and reduced tissue cadmium accumulation in rice via *T. pubescens* colonization

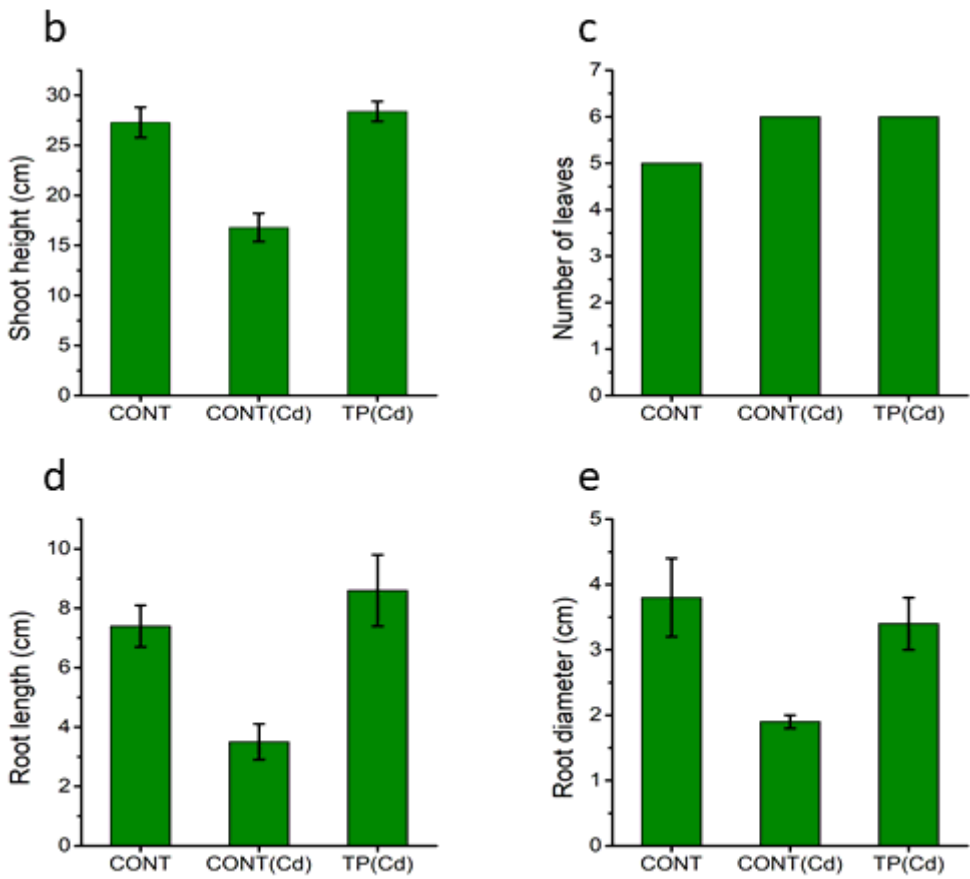
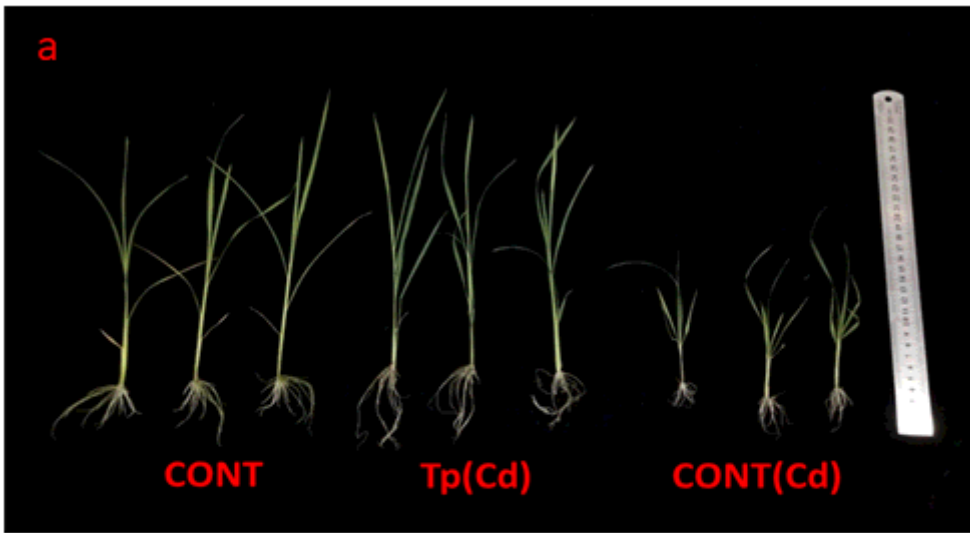


Figure 5

Effect of *T. pubescens* on growth of rice plants with high Cd stress.

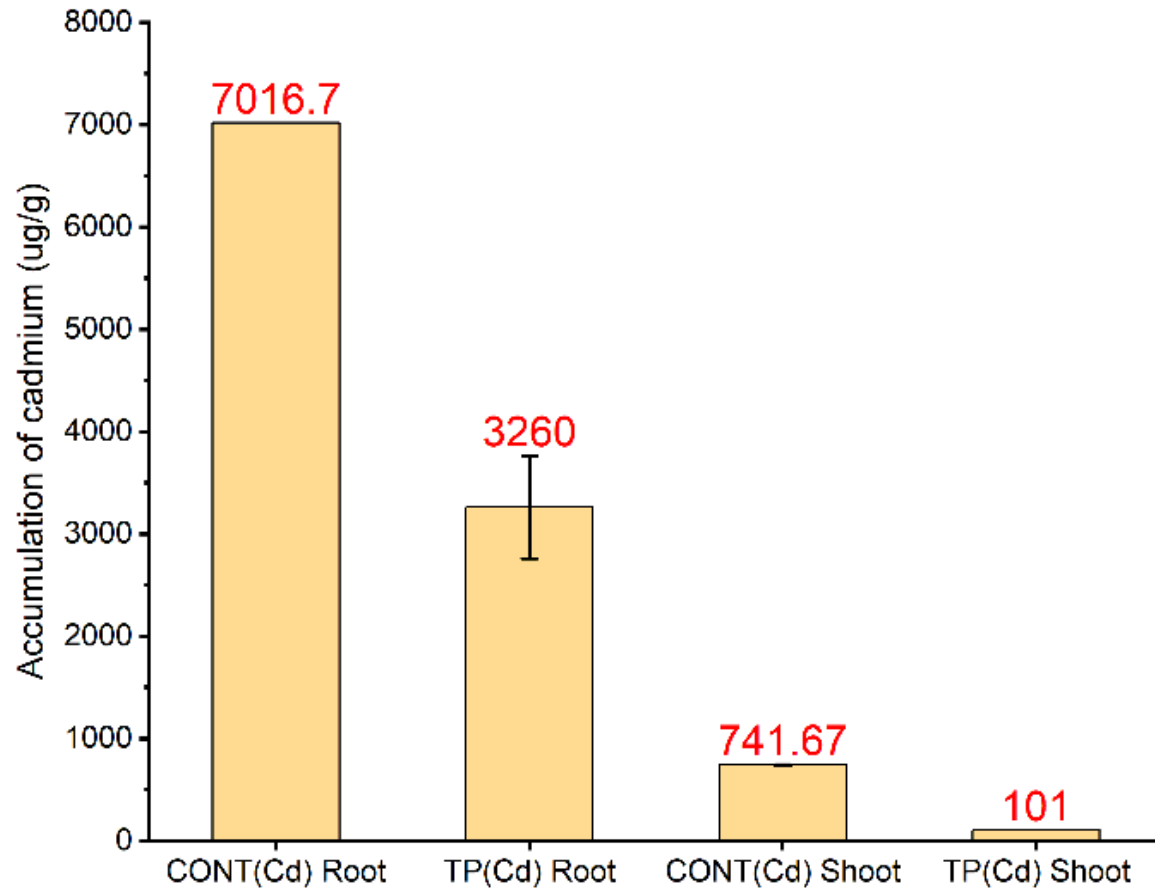


Figure 6

Effect of *T. pubescens* on Cd accumulation of rice plants under cadmium stress

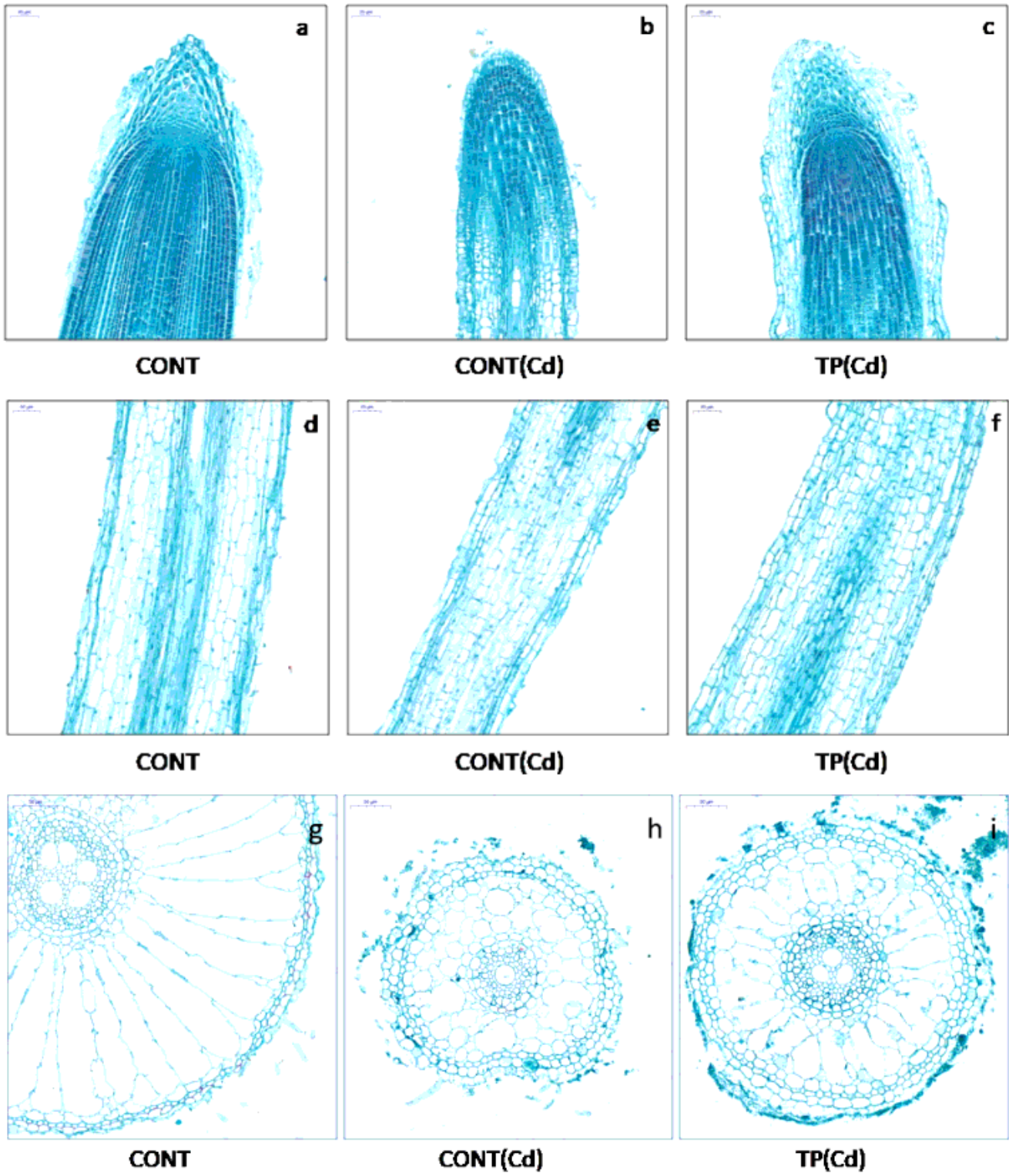


Figure 7

Effect of *T. pubescens* on rice root tissues under Cd toxicity.