

# Physiological and Ultrastructural Effects of Cadmium on Poplar (*Populus x euramericana*) Leaves

YUNQIU JIAO<sup>1,2</sup>, JINHUA ZOU<sup>1</sup>, WEI GE<sup>1,3</sup>, WUSHENG JIANG<sup>1</sup> AND DONGHUA LIU<sup>\*</sup>

<sup>1</sup> Tianjin Key Laboratory of Animal and Plant Resistance, College of Life Sciences, Tianjin Normal University, Tianjin 300387, P.R. China

<sup>2</sup> Tianjin No. 7 Junior and Senior high School, Tianjin 300160, P.R. China

<sup>3</sup> Key Laboratory of Bamboo and Rattan Science and Technology of the State Forestry Administration, International Centre for Bamboo and Rattan, Beijing 100102, P.R. China

\* Correspondence author: E-mail: donghua@mail.zlnet.com.cn (Donghua Liu)

Y. Jiao, J. Zou, W. Ge, W. Jiang and D. Liu. 2015. Physiological and Ultrastructural Effects of Cadmium on Poplar (*Populus x euramericana*) Leaves. *Baltic Forestry* 21(1): 106–113.

## Abstract

We investigated concentrations of soluble proteins, changes of photosynthetic (chlorophyll content, chlorophyll fluorescence), and ultrastructural characteristics in leaves of *Populus 2025* (*Populus x euramericana*) grown in Hoagland nutrient solution spiked with different concentrations of Cd (0, 50, 100 and 500  $\mu\text{M}$ ) for 40 days. Concentration of chlorophylls decreased in the groups treated with Cd when compared with control, and declined with prolonged duration of treatment in the most cases. The soluble protein content decreased at the 40<sup>th</sup> day of Cd treatments. The photosynthetic parameters  $F_v/F_m$ ,  $F_v2/F_m2$ ,  $\Phi\text{PSII}$ , ETR, qP decreased and qN increased. Analysis of the chloroplast ultrastructure showed that Cd induced senescence like symptoms, such as loosening and disorientation of the grana, dilation and degradation of the thylakoids and disruption of the outer membrane of the chloroplasts envelope. These ultrastructural changes observed in Cd-treated chloroplasts suggest that Cd probably induced premature senescence.

**Key words:** Chloroplast ultrastructure, Chlorophylls, Chlorophyll fluorescence parameters, Soluble proteins, *Populus*

## Introduction

Cadmium (Cd) is naturally found in low concentrations in the environment. It is a non-essential element and is released into the environment by power stations, heating systems, metal-working industries or urban traffic (Benavides et al. 2005). It is thought to be a particularly dangerous pollutant due to its high toxicity and good solubility in water (Pinto et al. 2004). Cd can be absorbed readily by plants and accumulated in the human body through the food chain (Järup and Åkesson 2009).

Phytoremediation by using vegetation to remove, detoxify, or stabilize heavy metals is a promising tool for decontamination of polluted soil and water. Poplars and willows have the potential to provide a cheap method of cleaning up Cd-contaminated soils because of their high biomass production and high bioaccumulation coefficient for Cd (Dickinson 2000, Robinson 2000). According to the results from ultrastructural changes in roots, ICP-AES investigation, oxidative stress of *Populus 2025* in the previous investigations, it can be regarded as an efficient phytoextracting plant with considerable ability to accumulate Cd (Cd concentration in shoot exceeding 0.01% (w/w) (Jiao et al. 2012).

Photosynthesis is especially sensitive to the presence of Cd in chloroplasts (for reviews see Sanità di Toppi and Gabbrielli 1999, Pietrini et al. 2005, Kummerová et al. 2010, Jiao et al. 2012). Siedlecka et al. (1997) and Seregin and Ivanov (2001) indicated that Cd could interfere with the whole photosynthetic process, from chlorophyll biosynthesis and degradation, assembly of pigment protein complexes and thylakoids, to the electron transport chain, the Calvin cycle enzymes and the sugar transport and consumption. Cd stress generally reduces plants growth due to reduction in chlorophyll content and consequent inhibition of photosynthesis (Han et al. 2006). The PSII reaction center in the leaf is damaged, leading to the inhibition of photosynthesis (Li et al. 2008). Some ultrastructural studies were reported, such as in maize (da Cunha et al. 2008), *Allium cepa* (Liu and Kottke 2004), *Arabidopsis thaliana* (Van Belleghem et al. 2007), *Sedum alfredii* (Jin et al. 2008), tomato (Gratao et al. 2009) and *Populus* (Cocozza et al. 2008, 2011, Jiao et al. 2012). However, few investigations on toxic effects of Cd on the structure of chloroplast in leaves of poplar trees exposed to Cd have been reported.

In the present work, we focused on the effects of Cd on chlorophyll content, chlorophyll fluorescence

and chloroplast structure of *Populus 2025* leaves, in order to establish an overall picture of the Cd toxicity syndrome at the structural and functional levels and to better understand the tolerance mechanism of *Populus* under Cd stress.

## Materials and Methods

### Plant material and growth conditions

Woody cuttings (20 cm long) from one year-old shoots of *Populus 2025* (*Populus×euramericana*) were rooted in vermiculite watered with 1/2 strength Hoagland's nutrient solution for a month. Then they were selected for uniformity of roots and new shoots, and transferred to full-strength Hoagland nutrient solution spiked with different concentrations of Cd (0, 50, 100 and 500  $\mu\text{M}$ ) and grown in plastic containers (8 L) for 40 days. Cadmium was provided as cadmium chloride ( $\text{CdCl}_2$ ). The nutrient solution consisted of 0.75 mM  $\text{K}_2\text{SO}_4$ , 0.65 mM  $\text{MgSO}_4$ , 0.01 mM KCl, 0.25 mM  $\text{KH}_2\text{PO}_4$ , 2 mM  $\text{Ca}(\text{NO}_3)_2$ , 100  $\mu\text{M}$  FeEDTA, 10  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 1  $\mu\text{M}$   $\text{MnSO}_4$ , 0.1  $\mu\text{M}$   $\text{CuSO}_4$ , 0.05  $\mu\text{M}$   $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$  and 1  $\mu\text{M}$   $\text{ZnSO}_4$ , adjusted to pH 5.5. The experiments were conducted in a greenhouse under a 14 h photoperiod at 26/20°C (day/night) and 65–75% humidity. The solutions were constantly aerated and replaced every 10 days. Plants cultivated without Cd were used as control. Any visible symptoms of Cd toxicity in the leaves and roots were noted. Five replicates for each treatment were prepared to give a total of 20 pots. Twenty-five seedlings grew each pot.

### Measurements of chlorophylls

Leaf samples from each treatment were homogenized in 5 ml of 80 acetone at 4°C, and 5 ml of acetone was added in each tube (10 ml total volume). Tubes were stored in the dark at 4°C for 12 h prior to measurements. The samples were centrifuged at 4000 rpm, and absorbance was measured at 646 and 663 nm with UV-Vis spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan). Chlorophyll *a* ( $C_a$ ) and chlorophyll *b* ( $C_b$ ) concentrations were calculated according to Zarco-Tejada et al. (2005):

$$C_a \text{ [mg/g fresh matter]} = [12.21 \cdot A_{663} - 2.81 \cdot A_{646}] \cdot \text{ml acetone/mg fresh matter}$$

$$C_b \text{ [mg/g fresh matter]} = [20.13 \cdot A_{646} - 5.03 \cdot A_{663}] \cdot \text{ml acetone/mg fresh matter}$$

### Measurements of chlorophyll fluorescence

Chlorophyll fluorescence quenching analysis was carried out at room temperature using LI 6400 (LI-6400, LI-COR, Lincoln, USA) equipped with a fluorescence unit. Leaves were darkened for 12 h prior to measurement. The minimum (dark) fluorescence ( $F_0$ ) was ob-

tained upon excitation of leaves with a weak beam. The maximum fluorescence ( $F_m$ ) was determined following a saturating red light (7200  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). Yield of variable fluorescence ( $F_v$ ) was calculated as  $F_m - F_0$ , and maximum efficiency of PSII photochemistry in the dark-adapted state as  $F_v/F_m = (F_m - F_0)/F_m$ . Efficiency of energy harvesting by open reaction centers of photosystem II for light-adapted leaves were calculated as:  $F'_v/F'_m = (F'_m - F'_0)/F'_m$ , where  $F'_0$  is minimal fluorescence of a momentarily darkened leaf, and  $F'_m$  is maximal fluorescence during a saturating flash light of  $>7 \text{ mmol m}^{-2} \text{s}^{-1}$ . Photochemical quenching ( $q_p$ ) was calculated as indicated by the manufacturer's manual for the LI-6400 leaf chamber fluorometer,  $q_p = (F'_m - F_s)/(F'_m - F'_0)$ , where  $F_s$  is the Chl fluorescence yield during actinic illumination. Other fluorescence parameters were measured on light adapted leaves using the equations of Genty et al. (1989) (quantum yield of PSII electron transport,  $\Phi_{\text{PSII}} = F'_m - F_s / F'_m$ ; apparent electron transport rate,  $\text{ETR} = (F'_m - F_s)/F'_m \times \text{PPFD} \times 0.5 \times 0.84$ ; non-photochemical quenching,  $q_N = 1 - (F'_m - F'_0)/(F'_m - F_0)$ ). For the calculation of ETR, PPFD was the photosynthetic photon flux density of actinic illumination, 0.5 was assumed as the fraction of the excitation energy distributed to PSII and 0.84 as the fractional light absorption of the leaf. These data were collected every 10 days.

### Measurement of soluble protein contents

Measuring soluble proteins content in this investigation was carried out according to Bradford's method (Bradford 1976) using bovine serum albumin (BSA) as a standard. The fresh leaves from each treatment (6 seedlings) were washed in distilled water, dried and put in a mortar with 5 mL 0.05 M phosphate buffer (PBS) (pH 7.8) at the end of each time interval (10 days) of the Cd treatment. The homogenate was centrifuged at 10,000 rpm for 20 min and the supernatant was used for analysis of the soluble protein content. The soluble protein content was expressed as mg per g of fresh weight.

### Transmission electron microscopy

The leaf samples of the middle area of leaf blades from control and the treated groups grown for 40 days were hand cut with a razor blade into 1 mm<sup>2</sup> pieces and fixed in a mixture of 2% formaldehyde and 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 2 h and then thoroughly washed with the same buffer three times. This was followed by post-fixation with 2% osmium tetroxide in the same buffer for 2 h. They were dehydrated in an acetone series, and embedded in Spurr's ERL resin (Spurr 1969). For ultrastructural observations, ultrathin sections of 75-nm thickness

were cut on an ultramicrotome (Leica EM UC6, Germany) with a diamond knife, and were mounted in copper grids with 300 square mesh. The sections were stained with 2% uranyl acetate for 50 min and lead citrate for 15 min. Observation and photography were accomplished by transmission electron microscopy (JEM-1230, Joel Ltd, Tokyo, Japan).

**Statistical analysis**

Each treatment was replicated 5 times for statistical validity. Analysis of variance of the data was done with SigmaPlot 8.0 software. For statistical analysis, one-way analysis of variance (ANOVA) and *t*-test were used to determine the significance at *P* < 0.05.

**Results**

**Effects of Cd on contents of leaf chlorophylls**

The contents of chlorophyll *a*, *b* and *a+b* of *Populus 2025* during the whole treatment were presented in Figure 1. Cadmium caused the contents of leaf chlorophylls in *Populus 2025* to decrease significantly (*P* < 0.05) when compared with control, except for chlorophyll *b* exposed to 100 μM Cd at the 20<sup>th</sup> day. The contents of chlorophylls at 500 μM Cd were noted to be low significantly (*P* < 0.05) in comparison with control and the other treatment groups after the 20 to 30 day of treatment. The contents of chlorophyll *a*, *b* and *a+b* at 50 μM and 100 μM Cd showed increased trend during 10-20 days and then declined with pro-

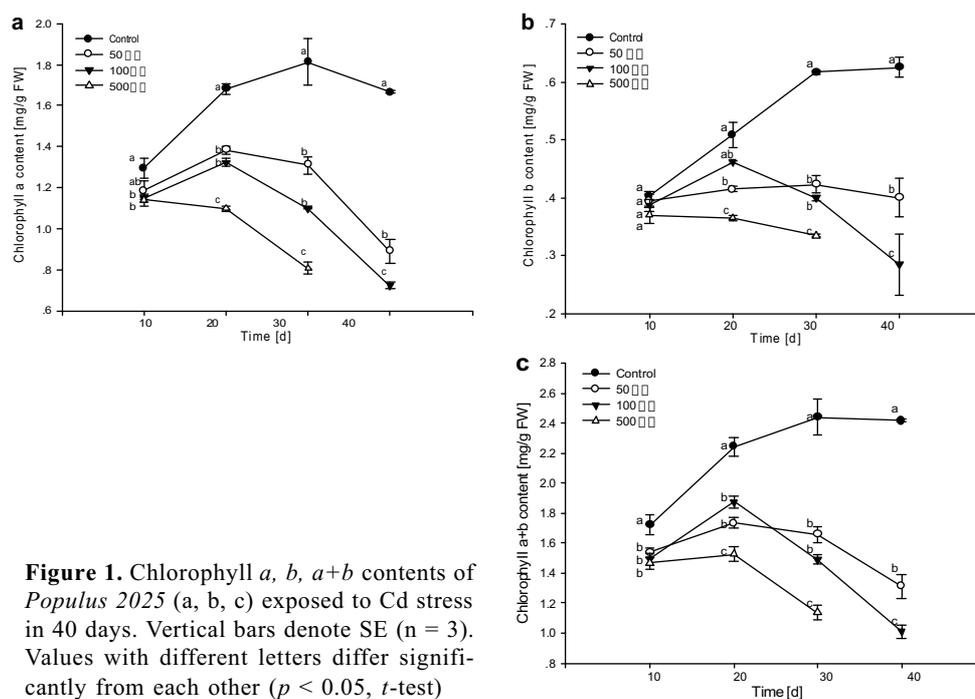
longing the treatment time, except for chlorophyll *b* at 50 μM Cd at the 30<sup>th</sup> day.

**Measurement of chlorophyll fluorescence characteristics**

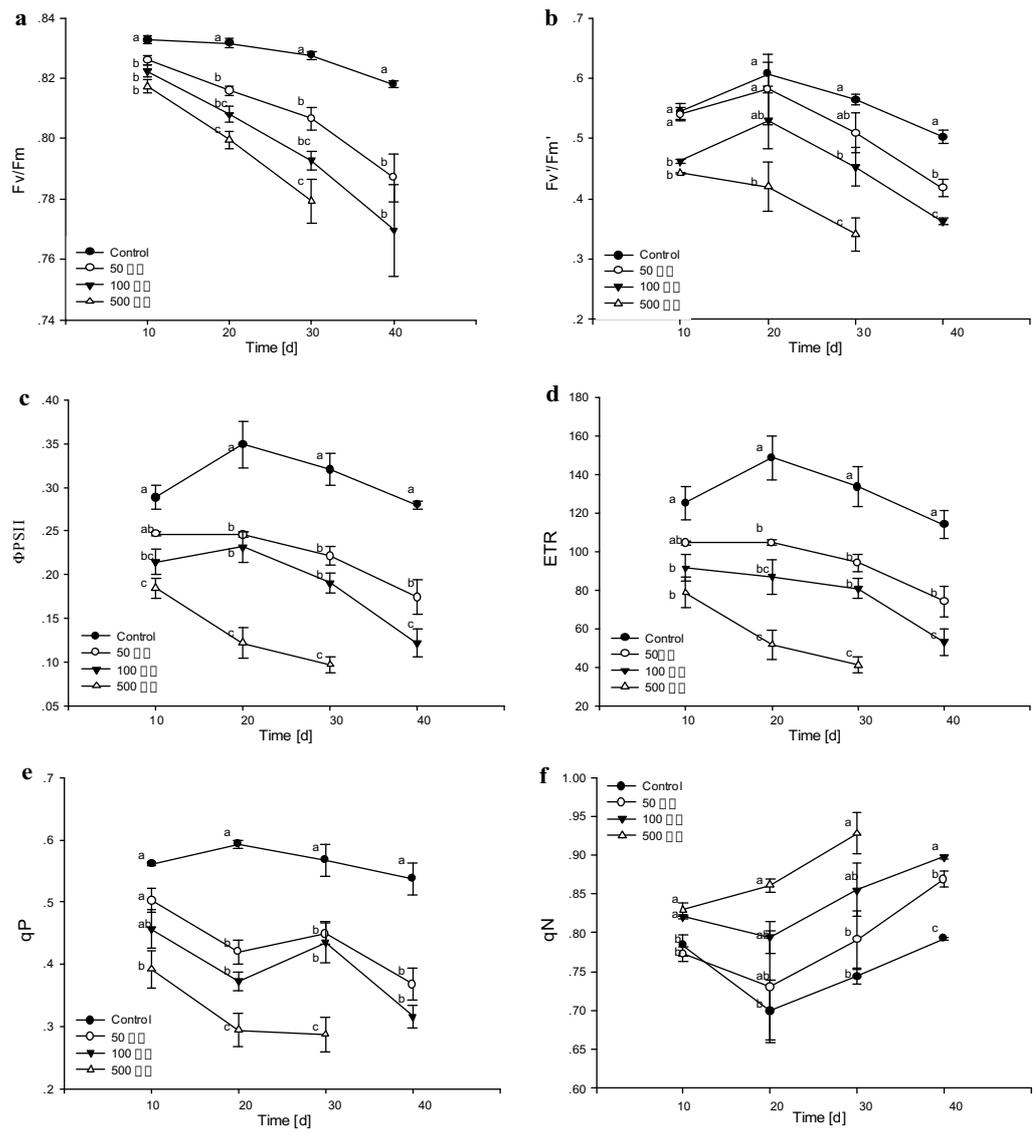
Figure 2a showed the changes of fluorescence parameters in dark-adapted leaves.  $F_v/F_m$  in *Populus 2025* exposed to 50 μM-500 μM Cd was lower than that in control and represented a decreased tendency with prolonging the treatment time.  $F_v/F_m$  in *Populus 2025* exposed to 50 μM Cd was more or less the same as 100 μM treatment during 40 days. Figure 2b-f showed the changing of fluorescence parameters in light-adapted leaves. Decrease tendency in photochemical efficiency of PSII ( $F_v'/F_m'$ ), actual photochemical efficiency of PSII ( $\Phi$ PSII), electron transfer rate (ETR) and photochemical quenching (qP) and increase tendency of qN were observed.  $F_v'/F_m'$  of *Populus 2025* exposed to 100 μM and 500 μM Cd was significantly lower (*P* < 0.05) in comparison with control, except for 100 μM Cd treatment for 20 days. Significant difference (*P* < 0.05) was shown in  $\Phi$ PSII, ETR and qP at 50 μM to 100 μM Cd after 20 days when compared with control. Significant increase of qN (*P* < 0.05) was found in the treatment group exposed to 500 μM Cd when compared with control.

**Effects of Cd on soluble protein contents**

The effects of Cd on soluble protein contents are presented in Figure 3. The results indicated that



**Figure 1.** Chlorophyll *a*, *b*, *a+b* contents of *Populus 2025* (a, b, c) exposed to Cd stress in 40 days. Vertical bars denote SE (n = 3). Values with different letters differ significantly from each other (*p* < 0.05, *t*-test)

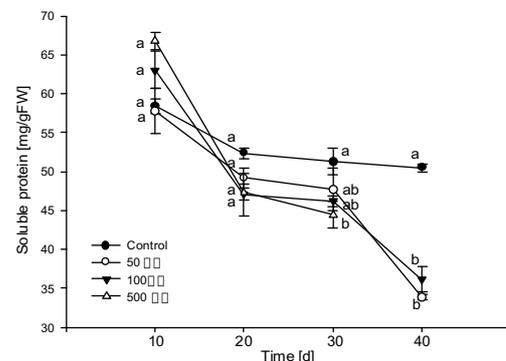


**Figure 2.** Effects of Cd on changes of  $F_v/F_m$ ,  $F_v'/F_m'$ ,  $\Phi_{PSII}$ , ETR,  $qP$ ,  $qN$  in *Populus 2025* treated with different concentrations of Cd in 40 days. Values with different letters differ significantly from each other ( $p < 0.05$ ,  $t$ -test).

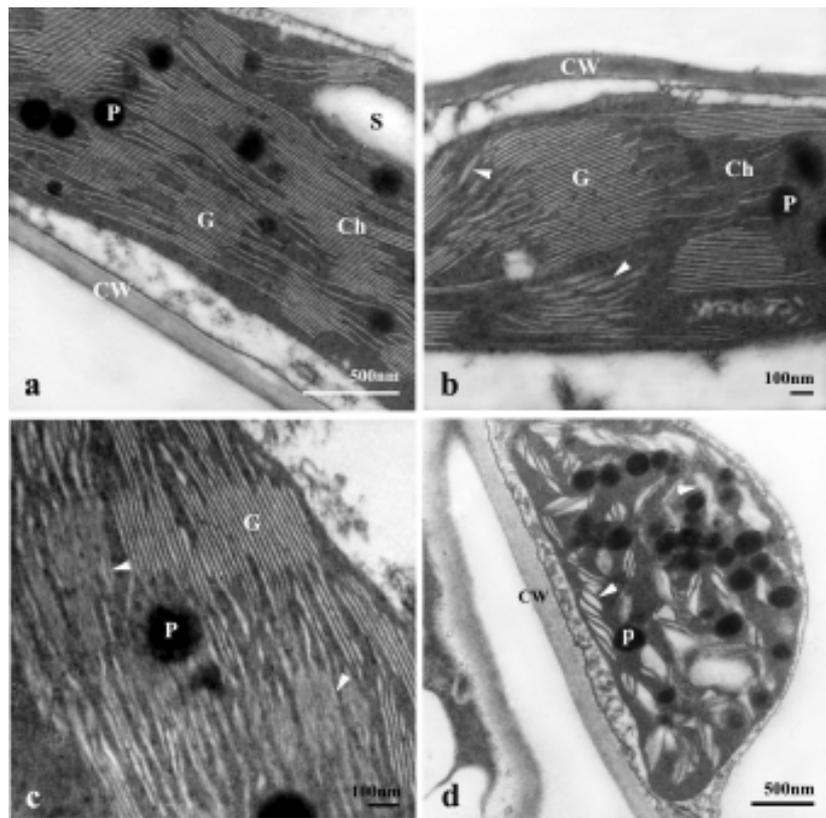
soluble protein contents in leaves declined after the 20 day of the treatment. At 500  $\mu M$  Cd, the content declined significantly ( $P < 0.05$ ) at the 30<sup>th</sup> day when compared with control and other treatment groups. The contents in all treatment groups decreased ( $P < 0.05$ ) in comparison with control at the 40<sup>th</sup> day.

**Chloroplast ultrastructure**

Transmission electron micrographs of the leaf cells of both control and Cd treated plants were shown. The chloroplasts of control plants were lens-shaped. They show a well-organized internal membrane structure with normally developed grana and stroma thylakoids (Figure 4a). In the cells of *Populus 2025* treated with Cd, almost all the chloroplasts were affected, but



**Figure 3.** Effects of various Cd concentrations on the contents of soluble protein in leaves of *Populus 2025* exposed to Cd stress in 40 days. Vertical bars denote SE ( $n = 3$ ). Values with different letters differ significantly from each other ( $p < 0.05$ ,  $t$ -test)



**Figure 4.** TEM micrographs of chloroplasts from control and Cd-treated of *Populus 2025*. a. Chloroplasts with a well-organized internal membrane structure with normally developed grana and stroma thylakoids in control cell. b. Slightly changed chloroplast in the plant exposed to 50  $\mu\text{M}$  Cd. Slightly disintegrated thylakoid membrane (arrows). c. Dissolved thylakoid membrane in the leaf treated with 100  $\mu\text{M}$  Cd (arrows). d. Dissolved grana (arrows) and more plastoglobuli in the chloroplasts exposed to 100  $\mu\text{M}$  Cd.

Abbreviations: Ch, chloroplast; CW, cell wall; G, grana stack; P, plastoglobuli; S, starch grain

to different degrees. In the comparison with control, some changes occurred in chloroplasts treated with 50  $\mu\text{M}$  Cd. E.g., some of thylakoid membranes were dilated (Figure 4b) in some cells. A disrupted outer membrane of the chloroplasts envelope was occasionally found. With the further increase in Cd concentration (100  $\mu\text{M}$ ), the toxic symptoms in chloroplasts were more significant because the thylakoid membrane was damaged and sometimes disappeared (Figure 4c). In some of seriously damaged chloroplasts, they seemed to be oval and the thylakoids were disorderly arranged. More than half of grana were dissolved (Figure 4d). The chloroplasts exposed to Cd contained more plastoglobuli than control cells (Figure 4d).

## Discussion

The results from the present investigation indicated that the amounts of chlorophyll *a*, chlorophyll *b* and consequently total chlorophylls were lower than that in control plants, which is in agreement with results of Pietrini et al. (2010) and Shen et al. (2010) in poplar. Nada et al. (2007) indicated that Cd could induce Fe deficiency in almond seedlings exposed to 25, 50, 100, 150  $\mu\text{M}$  Cd, which resulted in the decrease in ferredoxins necessary for the light-induced oxido-re-

duction process and reduction in chlorophyll content. In our previous investigation, large amounts of Cd were accumulated in the leaves (Jiao et al. 2012) and Cd could induce Fe deficiency in *Populus 2025* treated with 50  $\mu\text{M}$  and 100  $\mu\text{M}$  Cd (unpublished data). Hence, we indicated that Cd induced Fe deficiency and resulted in the decrease of chlorophyll content in the present investigation.

Chlorophyll fluorescence is widely used in analyzing the photosynthetic apparatus, detailed analysis of change in photosynthetic capacity, identifying injury to leaves in the absence of visible symptoms, understanding the mechanism of photosynthesis and the mechanism by which a range of environmental factors alter photosynthetic activity under both biotic or abiotic stresses (Sayed 2003). Chlorophyll fluorescence depends on to a great extent on pigment content and leaf ability to photosynthesis (Gitelson et al. 1999). The results in the present investigation indicated that  $F_v/F_m$ ,  $F_v'/F_m'$ ,  $\Phi\text{PSII}$ , ETR,  $qP$  decreased and  $qN$  increased. The decrease could be explained by the fact that Cd affected the reaction center of photosynthesis and disturbed the photosynthesis process (Maxwell and Johnson 2000). Pietrini et al. (2010) indicated that the peculiar Cd accumulation in small necrotic areas close to the main vein in willow

and poplar clones is associated with low values of the chlorophyll fluorescence parameters (Fo, Fm, Fv D Fm,  $\Phi$ PSII, qP and NPQ). The findings supported the results in the present investigation.

Soluble protein content in organisms is an important indicator of reversible and irreversible changes in metabolism and responds to a wide variety of stressor (Singh and Tewari 2003). The results indicated that the soluble protein content in leaves of poplar 2025 exposed to Cd decreased ( $P < 0.05$ ) in comparison with control at the 40<sup>th</sup> day. Manisha and Dhingra (2004) reported similar observations while working on pea. Siddhu and Ali Khan (2012) found that at higher concentration ( $10^{-2}$  M Cd), the protein content was inhibited. Our findings confirmed the studies by Steffens (1997) and Rolli et al. (2010). A decrease in protein content could be due to inactivation of protein synthesizing enzymes in the cell.

Simola (1977) did not observe any great changes in the fine structure under the effect of Cd on moss (*Spagnum memoreum*). Baszynski et al. (1980) indicated that Cd caused disorganization of grana and increased the number and size of plastoglobuli in tomato. The Cd-induced changes observed by electron microscopy in the chloroplast structure of *Populus* 2025 showed the same pattern as that observed in other plant species treated with Cd, and are similar to those found in senescent tissues (Barceló et al. 1988, Ouzounidou et al. 1997, Esposito et al. 2012). Chloroplasts from senescent leaves in the present investigation showed loosening and disorientation of the grana, dilation and degradation of the thylakoids and disruption of outer membrane of the chloroplasts envelope. The ultrastructural changes observed in chloroplasts of Cd-treated plants suggest that Cd probably induced premature senescence. Smart (1994) indicated that chloroplasts were one of the earliest sites of catabolism in leaf senescence. The increase in stroma volume is a common symptom in response to stress (Vitória et al. 2003, 2006). *Populus* 2025 treated with Cd exhibited swollen chloroplasts due to the increase in stroma volume. The effects of Cd on chloroplasts differ from those induced by other heavy metals. Nickel, cobalt, and copper induce accumulation of starch, probably by inhibition of vein loading (Rauser and Samarakoon 1980). In Cd-treated plants no starch accumulation was observed in the chloroplasts either in our experiment or by others (Barceló et al. 1988, Ouzounidou et al. 1997), which indicates that Cd either had no effect on vein loading or, more probably, Cd inhibited photosynthesis more intensively than the translocation of photoassimilates (Barceló et al. 1988, Ouzounidou et al. 1997, Djebali et al. 2005).

## Conclusion

*Populus* 2025 was selected for further research because it can be regarded as an efficient phytoextracting plant with considerable ability to accumulate Cd. Alterations in ultrastructure and chlorophyll fluorescence parameters would occur before any visible symptom of toxicity appears, and the endpoint based on these parameters might more sensitive or indicative than morphological observation in revealing ecotoxicity of Cd. Effects of Cd on them were comprehensively evaluated in this investigation focusing on chlorophyll content, soluble protein and chlorophyll fluorescence and ultrastructures in leaf cells. The information available in this work is an important step towards obtaining a better understanding of chlorophyll fluorescence parameters and ultrastructural changes caused by Cd. Chlorophyll fluorescence parameters is reduced and leaf ultrastructure is affected in *Populus* 2025 under Cd stress. Hence, more works are needed to analyze the potential of *Populus* 2025 to accumulate Cd from contaminated soils and the effects of Cd on photosynthesis and leaf ultrastructure in non-laboratory environments. Better understanding of the mechanistic details of Cd detoxification in *Populus* 2025 may lead to engineering of these plants to enhance their Cd phytoremediation capacity.

## Acknowledgments

This project was supported by the National Natural Science Foundation of China. The authors wish to express their appreciation to the reviewers for this paper.

## References

- Barceló, J., Vázquez, M.D. and Poschenrieder, C. 1988. Structural and ultrastructural disorders in cadmium-treated bush bean plants (*Phaseolus vulgaris*). *New Phytologist* 108: 37–49.
- Baszynski, T., Wajda, L., Krol, M., Wolinska, D., Krupa, Z. and Tukendorf, A. 1980. Photosynthetic activities of cadmium-treated tomato plants. *Physiologia Plantarum* 48: 365–370.
- Benavides, M.P., Gallego, S.M. and Tomaro, M.L. 2005. Cadmium toxicity in plants. *Brazilian Journal of Plant Physiology* 17: 21–34.
- Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248–254.
- Cocozza, C., Minnocci, A., Tognetti, R., Iori, V., Zacchini, M. and Scarascia, M.G. 2008. Distribution and concentration of cadmium in root tissue of *Populus alba* determined by scanning electron microscopy and energy-dispersive x-ray microanalysis. *i Forest* 1: 96–103.

- Cocozza, C., Maiuro, L. and Tognetti R. 2011. Mapping Cadmium distribution in roots of Salicaceae through scanning electron microscopy with x-ray microanalysis. *Journal of Biogeosciences and Forestry* 4: 113-120.
- da Cunha, K.P.V., do Nascimento, C.W.A., Pimentel, R.M.D. and Ferreira, C.P. 2008. Cellular localization of cadmium and structural changes in maize plants grown on a cadmium contaminated soil with and without liming. *Journal of Hazardous Materials* 160: 228-234.
- Dickinson, N.M. 2000. Strategies for sustainable woodland on contaminated soils. *Chemosphere* 41: 259-263.
- Djebali, W., Zarrouk, M., Brouquisse, R., Kahoui, S.E.I., Limam, F., Ghorbel, M.H. and Chaïbi W. 2005. Ultrastructure and lipid alterations induced by cadmium in tomato (*Lycopersicon esculentum*) chloroplast membranes. *Plant Biology* 7: 358-368.
- Esposito, S., Sorbo, S., Conte, B. and Basile, A. 2012. Effects of heavy metals on ultrastructure and HSP70s induction in the aquatic moss *Leptodictyum riparium* Hedw. *International Journal of Phytoremediation* 14: 443-455.
- Genty, B., Briantais, J.M. and Baker, N.R. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica Et Biophysica Acta-general Subjects* 99: 87-92.
- Gitelson, A.A., Buschmann, C. and Lichtenthaler, H.K. 1999. The chlorophyll fluorescence ratio F735/F700 as an accurate measure of the chlorophyll content in plants. *Remote Sensing of Environment* 69: 296-302.
- Gratao, P.L., Monteiro, C.C., Rossi, M.L., Martinelli, A.P., Peresc, L.E.P., Medicid, L.O., Leae, P.J. and Azevedo R.A. 2009. Differential ultrastructural changes in tomato hormonal mutants exposed to cadmium. *Environmental and Experimental Botany* 67: 387-394.
- Han, S.H., Lee, J.C., Oh, C.Y. and Kim, P.G. 2006. Alleviation of Cd toxicity by composted sewage sludge in Cd-treated Schmidt birch (*Betula schmidtii*) seedlings. *Chemosphere* 65: 541-546.
- Järup, L. and Åkesson, A. 2009. Current status of cadmium as an environmental health problem. *Toxicology and Applied Pharmacology* 238: 201-208.
- Jiao, Y.Q., Ge, W., Qin, R., Sun, B.L., Jiang, W.S. and Liu, D.H. 2012. Influence of cadmium stress on growth, ultrastructure and antioxidative enzymes in *Populus* 2025. *Fresenius Environmental Bulletin* 21: 1375-1384.
- Jin, X.F., Yang, X.E., Islam, E., Liu, D. and Mahmood, Q. 2008. Effects of cadmium on ultrastructure and antioxidative defense system in hyperaccumulator and nonhyperaccumulator ecotypes of *Sedum alfredii* Hance. *Journal of Hazardous Materials* 156: 387-397.
- Kummerová, M., Zezul, Š., Král'ov, K. and Masarovičová, E. 2010. Effect of zinc and cadmium on physiological and production characteristics in *Matricaria recutita*. *Biologia Plantarum* 54: 308-314.
- Li, M., Zhang, L.J. and Tao, L. and Li, W. 2008. Ecophysiological responses of *Jussiaea rapens* to cadmium exposure. *Aquatic Botany* 88: 347-352.
- Liu, D. and Kottke, I. 2004. Subcellular localization of cadmium in the root cells of *Allium cepa* by electron energy loss spectroscopy and cytochemistry. *Journal of Biosciences* 29: 329-335.
- Manisha, D. and Dhingra, H.R. 2004. Cadmium partitioning and seed quality in 2 varieties of pea and their hybrid as influenced by rhizospheric cadmium. *Indian Journal of Plant Physiology* 9: 15-20.
- Maxwell, K. and Johnson, G.N. 2000. Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany* 51: 659-668.
- Nada, E., Ferjani, B.A., Ali, R., Imed, B.R.B.M. and Makki, B. 2007. Cadmium-induced growth inhibition and alteration of biochemical parameters in almond seedlings grown in solution culture. *Acta Physiologiae Plantarum* 29: 57-62.
- Ouzounidou, G., Moustakas, M. and Eleftheriou, E.P. 1997. Physiological and ultrastructural effects of cadmium on wheat (*Triticum aestivum* L.) leaves. *Archives of Environmental Contamination and Toxicology* 32: 154-160
- Pietrini, F., Iannelli, M.A., Montanari, R., Bianconi, D. and Massacci, A. 2005. Cadmium interaction with thiols and photosynthesis in higher plants. In: A. Hemantaranjan, (Editor), *Advances in Plant Physiology*. Jodhpur, India, Scientific Publishers. Volume 8, p. 313-326.
- Pietrini, F., Zacchini, M., Iori, V., Pietrosanti, L., Bianconi, D. and Massacci, A. 2010. Screening of poplar clones for cadmium phytoremediation using photosynthesis, biomass and cadmium content analyses. *International Journal of Phytoremediation* 12: 105-120.
- Pinto, A.P., Mota, A.M., de Varennes, A. and Pinto, F.C. 2004. Influence of organic matter on the uptake of cadmium, zinc, copper and iron by sorghum plants. *Science of the Total Environment* 326: 239-247.
- Rausser, W.E. and Samarakoon, A.B. 1980. Vein loading in seedlings of *Phaseolus vulgaris* exposed to excess cobalt, nickel and zinc. *Plant Physiology* 65: 578-583.
- Robinson, B.H., Mills, T.M., Petit, D., Fung, L.E., Green, S.R. and Clothier, B.E. 2000. Natural and induced cadmium-accumulation in poplar and willow: Implications for phytoremediation. *Plant and Soil* 227: 301-306.
- Rolli, N.M., Suvarnakhandi, S.S., Mulgund, G.S., Ratageri, R.H. and Taranath, T.C. 2010. Biochemical responses and accumulation of cadmium in *Spirodela polyrhiza*. *Journal of Environmental Biology* 31: 529-532.
- Sanità di Toppi, L. and Gabrielli, R. 1999. Responses to cadmium in higher plants. *Environmental and Experimental Botany* 41: 105-130.
- Sayed, O.H. 2003. Chlorophyll fluorescence as a tool in cereal research. *Photosynthetica* 41: 321-330
- Seregin, I.V. and Ivanov, V.B. 2001. Physiological aspects of cadmium and lead toxic effects on higher plants. *Russian Journal Plant Physiology* 48: 523-544.
- Shen, D.Y., Yang, W.Q., Zhang, J., Zhou, L.Q. and Wu, F.H. 2010. Effects of cadmium on photosynthetic characters and biomass production of a poplar (*Populus deltoids* × *Populus nigra*). *Journal of Sichuan Agricultural University* 28: 61-66.
- Siddhu, G. and Ali Khan, M.A. 2012. Effects of cadmium on growth and metabolism of *Phaseolus mungo*. *Journal of Environmental Biology* 33: 173-179.
- Siedlecka, A., Krupa, Z., Samuelsson, G., Öquist, G. and Gardeström, P. 1997. Primary carbon metabolism in *Phaseolus vulgaris* plants under Cd/Fe interaction. *Plant Physiology and Biochemistry* 35: 951-957.
- Simola, L.K. 1977. The effect of lead, cadmium, arsenate and fluoride ions on the growth and fine structure of *Sphagnum nemoreum* in aseptic culture. *Canadian Journal of Botany* 55: 426-435.
- Singh, P.K. and Tewari, R.K. 2003. Cadmium toxicity induced changes in plant water relations and oxidative metabolism of *Brassica juncea* L. plants. *Journal of Environmental Biology* 24: 107-112.
- Smar, C.M. 1994. Gene expression during leaf senescence. *New Phytologist* 126: 419-448
- Spurr, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research* 26:31-43.

- Steffens, J.C.** 1997. The heavy metal binding peptides of plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 41: 553–575
- Van Belleghem, F., Cuypers, A., Semane, B., Smeets, K., Vangronsveld, J., d’Haen, J. and Valcke, R.** 2007. Subcellular localization of cadmium in roots and leaves of *Arabidopsis thaliana*. *New Phytologist* 173:495–508.
- Vitória, A.P., Da Cunha, M. and Azevedo, R.A.** 2006. Ultrastructural changes of radish leaf exposed to cadmium. *Environmental and Experimental Botany* 58: 47–52.
- Vitória, A.P., Rodrigues, A.P.M., Da Cunha, M., Lea, P.J. and Azevedo, R.A.** 2003. Structural changes in radish seedlings exposed to cadmium. *Biologia Plantarum* 47: 561–568.
- Zarco-Tejada, P.J., Berjón, A., López-Lozano, R., Miller, J.R., Martín, P., Cachorro, V., González, M.R. and De Frutos, A.** 2005. Assessing vineyard condition with hyperspectral indices: leaf and canopy reflectance simulation in a row-structured discontinuous canopy. *Remote Sensing of Environment* 99: 271–287.

Received 25 June 2014  
Accepted 29 January 2015