Effects of Thallium Stress on Photosynthesis, Chlorophyll Fluorescence Parameters and Antioxidant Enzymes Activities of *Coix Lacryma-jobi*

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Abstract: Levels of Tl (Thallium) in soil from 0 (control) to 50 $\mu\text{g/L}$ through 0.2, 0.5, 1 and 2.5 $\mu\text{g/L}$ were directly and positively correlated to levels of Tl in plant tissue, the accumulation being maximum in roots, intermediate in leaves and minimum in stems. Thallium, especially at higher concentrations, adversely affected photosynthesis (as judged based on chlorophyll fluorescence parameters), suggesting inhibition of photo-activation of PSII (Photosystems II), and also decreased the rate of photosynthesis, the rate of transpiration and stomatal conductivity drastically. Exposure to Tl also increased the activity of CAT (Catalase) (except at 1 $\mu\text{g/L}$) and POD (Peroxidase) (except at 0.2 $\mu\text{g/L}$), suggesting that the antioxidant systems in *Coix lacryma-jobi* were the main contributors of CAT and SOD (Superoxide Dismutase) and that the tolerance of *C. lacryma-jobi* to Tl is mainly due to this induced antioxidant machinery.

Key words: Antioxidant enzyme, *Coix lacryma-jobi* L., chlorophyll fluorescence, Thallium, wetland.

1. Introduction

Tl (Thallium) is a relatively rare and non-essential metal, which occurs mainly as monovalent and trivalent thallium oxide. Because it is highly toxic to animals, plants and microorganisms, Tl has attracted increasing concern [1]. Extensive mining of ore that contains Tl, smelting and burning of Tl-containing fossil fuels are the major sources of anthropogenic dispersion of Tl in the environment [2]. Although we now have some understanding of the environmental geochemistry and ecotoxicology of Tl, the mechanism of Tl toxicity is not entirely clear.

Because Tl^+ and K^+ have similar ionic radii [3], they can interfere with Na^+/K^+ ATPase and pyruvate kinase and induce oxidative stress in plants [2]. Thus, plants can readily accumulate Tl^+ from soil, and Tl thus enters the food chain and, in turn, the bodies of animals and human beings. In general, plants accumulate Tl mainly

in leaves and roots and to some extent in stems and fruits, whose extent and the pattern of accumulation being dependent on the species and on soil characteristics [4, 5]. Plant cells contain considerable amounts of K^+ in the chloroplasts, and Tl pollution can often lead to the accumulation of Tl in the chloroplasts, which may lead to physiological effects on photosynthesis and the activity of antioxidant enzymes because Tl^+ can interfere with Na^+/K^+ ATPase, pyruvate kinase and membrane phospholipids [2]. It is therefore important to understand the effect of Tl on the physiological parameters and antioxidant enzymes activity in plants. Unfortunately, there is little research in this area.

Coix lacryma-jobi L. or Job's tears, is an andropogonoid grass native to tropical Asia that is widely adventive and considered invasive [6]. The species is widely cultivated as a food plant and as a medicinal plant in East Asia and Southeast Asia. Earlier studies on the species were focused mainly on its biological characteristics, medicinal components

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and their efficacy, and on its genetics and genomics [7-11]. Despite being widely cultivated, little is known about the effects of Tl pollution on this plant. Therefore, the objective of the present study was to monitor the responses of chlorophyll fluorescence, photosynthetic parameters and the activity of antioxidant enzymes in *C. lacryma-jobi* to different levels of Tl stress and also to highlight the uptake and translocation of Tl in the plant.

2. Material and Methods

2.1 Test Soil and Thallium Concentrations

The test soil was a red clay. Surface soil (0-20 cm) and water were sampled from the garden of the Guangxi Institute of Botany, and the basic physiochemical properties of the samples were analyzed (Table 1). Plastic containers (tackle boxes, each 35 cm in diameter and 50 cm tall) were filled with the test soil (5 kg of soil in each box) and 4 L of water was added to each box. The treatments comprised a control (no added Tl) and thallium chloride to give five concentrations of Tl, namely 0.2, 0.5, 1, 2.5 and 50 µg/L.

2.2 Culture and Harvest for Plant

During the growing period of *C. lacryma-jobi*, the height of water was always maintained at 4 L of water in soil. After being cultivated for 120 days, chlorophyll fluorescence parameters, photosynthetic gas exchange parameters, and the activity of antioxidant enzymes were determined. After that, the plants were harvested; carefully washed with tap water and deionized water; separated into leaves, stems and roots; the parts cut into bits with stainless steel scissors; and dried at 40 °C for 48 h for elemental analysis. Total Tl in the plant material was estimated, following the protocol of Srivastava and D'Souza [12], after digesting the

oven-dried samples (100 mg each).

2.3 Photosynthetic Parameters

Chlorophyll fluorescence parameters and photosynthetic gas exchange parameters were determined by the method described by Lichtenthaler [13] using a LI-6400XT portable photosynthesis system (Li-Cor, Inc., Lincoln, NE, USA) and a portable fluorometer (Monitoring-PAM, Walz, Germany) separately. The rate of photosynthesis (Pn), the rate of transpiration (Tr), intercellular concentration of CO₂ (Ci) and stomatal conductivity (Gs) were measured from the middle region of the topmost fully expanded leaf at 25 °C under a light intensity of 1,200 µmol·m⁻²·s⁻¹, relative humidity of 40%, and CO₂ concentration of 370 µmol·mol⁻¹. The topmost fully expanded leaves of treated and control plants were first light- and dark-adapted for 20 min to obtain F and Fo. The values of Fm' and Fm (maximum fluorescence yield of light and dark-adapted leaves, respectively) were calculated with a saturation pulse, and the maximum photosystem II quantum yield was then calculated using the following formula: (Fm – Fo)/Fm = Fv/Fm. The effective quantum yield of PSII (Photosystems II), or Y(II), was calculated, as suggested by Genty [14], using the following formula: Y(II) = (Fm – F)/Fm. All measurements were the averages from five plants in each replication and were recorded during 8:00-11:00 a.m.

2.4 Antioxidant Enzymes Activities

The activities of SOD, POD and CAT were assayed by following the protocols of Asthir [15] with a slight modification. Leaves (samples of 0.3 g each) were homogenized in 5 cm³ of ice-cold 50 mM phosphate buffer (pH 6.5 for POD and SOD and 7.5 for CAT).

Table 1 Basic physiochemical properties of tested soil and water.

Soil					Water				
PH	TN (g/kg)	TP (g/kg)	TK (g/kg)	T-Tl (mg/kg)	PH	DO (mg/L)	SpC (µs/cm)	Ca ²⁺ (mg/L)	T-Tl (µg/L)
6.50	1.17	0.80	0.78	0.002	7.38	1.56	235.00	53.00	0.005

Table 2 Concentrations of Tl in dry giant reed plants after 4-month cultivation.

Concentration (µg/L)	Leaves (mg/kg)	Shoots (mg/kg)	Roots (mg/kg)
0.00	0.02**	0.03**	0.33*
0.20	0.19**	0.20*	0.62*
0.50	1.32**	0.42*	2.13**
1.00	2.13*	0.66*	3.48**
2.50	2.46*	1.35**	4.09**
50.00	3.26**	2.08**	5.23**

Note: Data with a single star (*) indicate a significant difference at $P < 0.05$ among different Tl treatments in the same part of plants, and with double stars (**) indicate a significant difference at $P < 0.001$.

The extracts were centrifuged at 10,000 g for 20 min at 0-4 °C in a Beckmann refrigerated centrifuge, and the supernatants were used for the enzyme activity assays.

2.5 Data Analysis

All data were statistically analyzed using SPSS (Statistical Product and Service Solutions). The accumulated amounts of Tl were expressed as mean $\pm$ SD (Standard Deviation) of four replicates, and ANOVA (Analysis of Variance) was applied to assess significant differences ($P < 0.05$, unless stated otherwise), if any, among the various treatments.

3. Results and Discussion

3.1 Accumulation of Tl

In ascending order of the amount of Tl accumulated, the plant parts were stems, leaves and roots, and the accumulation was positively correlated to the Tl concentration (Table 2). The maximum accumulation in roots occurred at 50 µg/L of Tl and its translocation factor was up to 1.02, the actual amounts being 2.08 mg/kg in stems, 3.23 mg/kg in leaves and 5.23 mg/kg in roots (Table 2). Earlier reports indicate that when grown on Tl-contaminated soils, many crops, such as *Brassica napus*, *Triticum turgidum* and *Biscutella laevigata*, may contain Tl at concentrations above the maximum permissible limits [5, 16, 17]. Compared to these species, the TF of *C. lacryma-jobi* was higher, indicating that *C. lacryma-jobi* can accumulate more Tl than these species because it has a higher capacity

for Tl^+ uptake, given that the uptake of the chemically similar Tl^+ and K^+ are linked [5, 18].

3.2 Photosynthetic Parameters

The relative chlorophyll content of *C. lacryma-jobi* varied with Tl concentration, but the only statistically significant difference was observed between the 50 µg/L treatments and 0, 0.2, 1 or 2.5 µg/L treatments (Fig. 1). Chlorophyll fluorescence parameters showed that Tl, especially at higher concentrations, affected photosynthesis adversely (Fig. 1). At 50 µg/L, Tl not only decreased the relative chlorophyll content but also decreased the primary photochemical efficiency of PSII (Fv/Fm), Fv/Fo and yield. These results indicated that PSII reaction centers were seriously damaged at Tl concentrations of 50 µg/L and that Tl at that concentration inhibited the photo-activation of PSII because a decline in Fv/Fm and Fv/Fo indicated a disturbance in or damage to the photosynthetic apparatus [19, 20]. One possible reason for the reduced chlorophyll fluorescence in the present study is a functional disorder in antenna complexes that increased Fo and thereby decreased Fv/Fm and Fv/Fo and in turn, photosynthesis [21]. At the same time, once the structure of PSII was destroyed, photosynthesis decreased, and the light energy absorbed by leaves could not be converted into chemical energy, the result being inhibition of the initial photosynthesis reaction [22].

The stress induced by Tl decreased the Pn, Tr and Gs in the leaves drastically as compared to the control, but increased the Ci at 0.2 µg/L and 50 µg/L (Fig. 2).

Fig. 1 Chlorophyll fluorescence parameters of *C. lacryma-jobi* in the five concentrations of Tl treatments. Different lowercase letters on the top of the bars denote significant differences ($P < 0.05$) among different Tl treatments.

Fig. 2 Gas exchange parameters of *C. lacryma-jobi* under different level Tl conditions. Different lowercase letters on the top of the bars denote significant differences ($P < 0.05$) among different Tl treatments.

Fig. 3 Effect of Tl stress on SOD, COD and CAT activities in leaf of *C. lacryma-jobi*. Different lowercase letters on the top of the bars denote significant differences ($P < 0.05$) among different Tl treatments.

Two earlier studies indicated that one of the main reasons for heavy-metal toxicity came from the disturbed water relations in plants [23, 24]. Therefore, the authors propose that the decrease in P_n is due to the inhibition of various steps in the Hill reaction, the Calvin cycle and CO_2 fixation [24, 25]. At the same time, the C_i was significantly increased at 0.2 $\mu\text{g/L}$ and 50 $\mu\text{g/L}$ (Fig. 2), probably as the combined effect of stomatal conductance, chlorophyll content, and functioning of the photosynthetic apparatus—reduction in the rate of CO_2 exchange cannot be explained fully by any single factor [26].

3.3 Antioxidant Enzymes Activities

In plants exposed to Tl, the activity of CAT (except at 1 $\mu\text{g/L}$) and of COD (except at 0.2 $\mu\text{g/L}$) increased significantly compared to that in the control. However, POD activity was maximum at 1 $\mu\text{g/L}$ and minimum

at 50 $\mu\text{g/L}$ Tl (Fig. 3). In general, antioxidant enzymes are the common means in plants to regulate the ROS produced during metabolic processes [27]. Authors' results show that the activity of CAT and SOD increased under Tl stress, suggesting that the conversion of H_2O_2 increases under Tl stress, given that CAT converts H_2O_2 into water and molecular O_2 (Oxygen) and catalyzes the dismutation of O_2^- to H_2O_2 and O_2 . One possible reason is that Tl stress reduces the capacity of *C. lacryma-jobi* to assimilate carbon, thereby triggering an increase in photosynthetic electron flux to molecular oxygen, resulting in increased production of superoxide, namely hydrogen peroxide [28]. The activity of POD increased only at 1 $\mu\text{g/L}$, suggesting that it is not the most important H_2O_2 -scavenging enzyme in *C. lacryma-jobi*. And the activity of POD decreased at 50 $\mu\text{g/L}$, suggesting that POD can be damaged if exposed to a high level of Tl stress.

4. Conclusion

Taken together, these results show that the accumulation of Tl in leaves, stems and roots of *C. lacryma-jobi* increased with increase in Tl concentrations in soil-water, pointing to the greater capacity of the species for K⁺ uptake. The variation in photosynthetic parameters, including relative chlorophyll content, chlorophyll fluorescence parameters and photosynthetic gas exchange parameters, suggests that photo-activation of PSII was inhibited by Tl toxicity. However, the antioxidant systems in *C. lacryma-jobi*, mainly CAT and SOD, moderated the adverse effects of Tl—and, in turn, those of reactive oxygen species—on *C. lacryma-jobi*, because Tl triggers the activation of those systems.

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References

- [1] Gomez-Gonzalez, M. A., Garcia-Guinea, J., Labor, F., and Garrido, F. 2015. "Thallium Occurrence and Partitioning in Soils and Sediments Affected by Mining Activities in Madrid Province (Spain)." *Science of the Total Environment* 536 (12): 268-78.
- [2] Siegel, B. Z., and Siegel, S. M. 1976. "Effect of Potassium on Thallium Toxicity in Cucumber Seedlings: Further Evidence for Potassium-Thallium Ion Antagonism." *Bioinorganic Chemistry* 6 (4): 341-5.
- [3] Belowitz, R., and Donnell, M. J. O. 2013. "Ion-Selective Microelectrode Measurements of Tl (+) and K (+) Transport by the Gut and Associated Epithelia in *Chironomus Riparius*." *Aquatic Toxicology* 138-9 (8): 70-80.
- [4] Alshaal, T., Domokos-Szabolcsy, E., Marton, L. J., Katai-Balogh, P., Elhawat, N., El-Ramady, H., et al. 2013. "Phytoremediation of Bauxite-Derived Red Mud by Giant Reed." *Environmental Chemistry Letters* 11 (3): 295-302.
- [5] Renkema, H., Koopmans, A., Hale, B., and Berkelaar, E. 2015. "Thallium and Potassium Uptake Kinetics and Competition Differ between Durum Wheat and Canola." *Environmental Science and Pollution Research* 22 (3): 2166-74.
- [6] Leseberg, C. H., and Duvall, M. R. 2009. "The Complete Chloroplast Genome of *Coix lacryma-jobi* and a Comparative Molecular Evolutionary Analysis of Plastomes in Cereals." *Journal of Molecular Evolution* 69 (4): 311-8.
- [7] Han, Y., Wang, G., Liu, Z., Liu, J., Yue, W., Song, R., et al. 2010. "Divergence Incentromere Structure Distinguishes Related Genomes in *Coix lacryma-jobi* and its Wild Relative." *Chromosoma* 119 (1): 89-98.
- [8] Woo, J. H., Dapeng, L., Orita, H., Coulter, J., Tully, E., Kwon, T. K., et al. 2007. "Coix Seed Extract, A Commonly Used Treatment for Cancer in China, Inhibits NFκB and Protein Kinase C Signaling." *Cancer Biology & Therapy* 6 (12): 2005-11.
- [9] Lu, Y., Zhang, B., Jia, Z., Wu, W., and Lu, Z. 2011. "Hepatocellular Carcinoma HepG2 Cell Apoptosis and Caspase-8 and Bcl-2 Expression Induced by Injectable Seed Extract of *Coix lacryma-jobi*." *Hepatobiliary & Pancreatic Diseases International* 10 (3): 303-7.
- [10] Cai, Z. X., Liu, H. J., Xie, W. J., and Jin, W. W. 2013. "Research Advances in *Coix L.* Biology." *Crops* 2013 (2): 16-22.
- [11] Cai, Z. X., Liu, H. J., He, Q. Y., Pu, M. W., Chen, J., Lai, J. S., et al. 2014. "Differential Genome Evolution and Speciation of *Coix lacryma-jobi* L. and *Coix aquatica* Roxb. Hybrid Guangxi Revealed by Repetitive Sequence Analysis and Fine Karyotyping." *BMC Genomics* 15 (1): 1025.
- [12] Srivastava, S., and D'Souza, S. F. 2010. "Effect of Variable Sulfur Supply on Arsenic Tolerance and Antioxidant Responses in *Hydrilla verticillata* (L.f.) Royle." *Ecotoxicology and Environmental Safety* 73: 1314-22.
- [13] Lichtenthaler, H. K., Langsdorf, G., Lenk, S., and Buschmann, C. 2005. "Chlorophyll Fluorescence Imaging of Photosynthetic Activity with the Flash-Lamp Fluorescence Imaging System." *Photosynthetica* 43 (3): 355-69.
- [14] Asthir, B., Koundal, A., Bains, N. S., and Mann, S. K. 2010. "Stimulation of Antioxidative Enzymes and Polyamines during Stripe Rust Disease of Wheat." *Biologia Plantarum* 54 (2): 329-33.
- [15] Shah, K., and Nahakpam, S. 2012. "Heat Exposure Alters the Expression of SOD, POD, APX and CAT Isozymes and Mitigates Low Cadmium Toxicity in Seedlings of Sensitive and tolerant Rice Cultivars." *Plant Physiology*

and *Biochemistry* 57 (3): 106-13.

- [16] Pavličková, J., Zbiral, J., Smatanová, M., Houserová, P., Čížmárová, E., Havliková, S., et al. 2005. "Uptake of Thallium from Artificially and Naturally Contaminated Soils into Rape (*Brassica napus* L.)." *Journal of Agricultural and Food Chemistry* 53 (8): 2867-71.
- [17] Pošćić, F., Marchiol, L., and Schat, H. 2013. "Hyperaccumulation of Thallium is Population-Specific and Uncorrelated with Caesium Accumulation in the Thallium Hyperaccumulator *Biscutella laevigata*." *Plant and Soil* 365 (1): 81-91.
- [18] Tremel, A., Masson, P., Sterckeman, T., Baize, D., and Mench, M. 1997. "Thallium in French Agrosystems—I. Thallium Contents in Arable Soils." *Environmental Pollution* 95 (3): 293-302.
- [19] Li, S. L., Yang, W. H., Yang, T. T., Chen, Y., and Ni, W. Z. 2015. "Effects of Cadmium Stress on Leaf Chlorophyll Fluorescence and Photosynthesis of *Elsholtzia argyi*-A cadmium Accumulating Plant." *International Journal of Phytoremediation* 17 (1-6): 85-92.
- [20] Wang, Y., Chai, L. Y., Yang, Z. H., Mubarak, H. S., and Tang, C. J. 2016. "Chlorophyll Fluorescence in Leaves of *Ficus tikoua* Under Arsenic Stress." *Bulletin of Environmental Contamination and Toxicology* 97 (4): 576-81.
- [21] Wang, L., Xu, Y. M., Sun, Y. B., Liang, X. F., and Lin, D. S. 2014. "Identification of Pakchoi Cultivars with Low Cadmium Accumulation and Soil Factors that Affect Their Cadmium Uptake and Translocation." *Frontiers of Environmental Science & Engineering* 8 (6): 877-87.
- [22] Liu, H. G., Hu, C. X., Sun, X. H., Tan, Q. L., Nie, Z. J., and Hu, X. M. 2010. "Interactive Effects of Molybdenum and Phosphorus Fertilizers on Photosynthetic Characteristics of Seedlings and Grain Yield of *Brassica napus*." *Plant and Soil* 326 (1): 345-53.
- [23] Vassilev, A., Vangronsveld, J., and Yordanov, I. 2002. "Cadmium Phytoextraction Present State Biological Backgrounds Research Needs." *Bulgarian Journal of Plant Physiology* 28 (3-4): 68.
- [24] Haouari, C. C., Nasraoui, A. H., Bouthour, D., Houda, M. D., Daieb, C. B., Mnai, J., et al. 2012. "Response of Tomato (*Solanum lycopersicon*) to Cadmium Toxicity: Growth, Element Uptake, Chlorophyll Content and Photosynthesis Rate." *African Journal of Plant Science* 6 (1): 1-7.
- [25] Dong, J., Wu, F., and Zhang, G. 2005. "Effect of Cadmium on Growth and Photosynthesis of Tomato Seedlings." *Journal of Zhejiang University, Science B* 6 (10): 974-80.
- [26] Ainsworth, E. A., and Rogers, A. 2000. "The Response of Photosynthesis and Stomatal Conductance to Rising [CO₂]: Mechanisms and Environmental Interactions." *Plant, Cell & Environment* 30 (3): 258-70.
- [27] Liu, N., Lin, Z., Guan, L., Gaughan, G., and Lin, G. 2014. "Antioxidant Enzymes Regulate Reactive Oxygen Species during Pod Elongation in *Pisum sativum* and *Brassica chinensis*." *Plos One* 9: e87588.
- [28] Odjegba, V. J., and Fasidi, I. O. 2007. "Changes in Antioxidant Enzyme Activities in *Eichhornia crassipes* (Pontederiaceae) and *Pistia stratiotes* (Araceae) under Heavy Metal Stress." *Revista De Biologia Tropical* 55 (55): 815-23.