Effects of Thallium stress on photosynthesis, chlorophyll fluorescence parameters and antioxidant enzymes activities of Arundo donax

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Abstract: We studied the influence of soil-water Thallium (TI) pollution on the seedling leaves photosynthesis, chlorophyll fluorescence parameters and antioxidant enzymes activities based on the energy grass species Arundo donax L. The soil-water TI separately set 0 (CK), 0.2, 0.5, 1, 2.5 and 50 µg/L. The amount of TI accumulation in leaves, stems and roots of A. donax increased with increasing TI (from 0 to 2.5 µg/L), and was still higher under high level TI (50 µg/L) than lower level TI (<2.5 µg/L). However, high level TI stress (50 µg/L) was significantly inhibited chlorophyll synthesis, and thus reduced the primary photochemical efficiency of PSII (Fv/Fm), potential activity of PSI (Fv/Fo), apparent quantum (Yield). Meanwhile, TI application mainly negatively influenced various photosynthetic parameters like Pn, Tr and Gs and SOD activity. Nevertheless, intercellular CO2 concentration (Ci) showed a contrary trend with Pn due to the effect of nonsotmal factors, and POD and CAT increased under high lever TI stress, showing H2O2 converts increased after 4-month growing of A. donax. This study suggests that A. donax was a tolerant plant species to TI may be mainly through induced antioxidant machinery.

Keywords Arundo donax, antioxidant enzymes, chlorophyll fluorescence, Thallium

INTRODUCTION
Thallium (TI) is a relatively rare and non-essential metal, mainly in the form of monovalent and trivalent thallium oxide. As its high toxicity to animals, plants, and microorganisms, TI has attracted increasing concerns (Gomez-Gonzaleza et al. 2015). Extensive TI-containing ore mining, smelting, and TI-containing fossil fuel burning are the major sources of anthropogenic dispersion of TI in the environment (Siegel and Siegel et al. 1976). Although there has been some progress in TI environmental geochemistry and ecotoxicology, the toxic mechanism of TI is not entirely clear.

As TI+ and K+ have similar ionic radii (Belowitz et al. 2013), it can interfere with Na+/K+ ATPase and pyruvate kinase and induce oxidative stress to plants (Siegel and Siegel et al. 1976). Thus, TI+ can be readily accumulated by plants from soil and through the food chain to enter the animal and human body. In general, plants accumulated thallium mainly in the leaves and roots and then in the stems and fruits, and the degree to which this occurs was species and soil characteristics dependent (Alshaal et al. 2013; Renkema et al. 2015). It is well known that in plant cells, there is a considerable portion of K+ in chloroplasts. TI pollution can often lead to the accumulation of TI in the chloroplasts of plants, which would result in the physiological effects of photosynthesis, antioxidant enzymes activities since TI+ can interfere with Na+/K+ ATPase, pyruvate kinase and membrane phospholipids (Siegel and Siegel et al. 1976). Thus, it is important to understand the effect of TI on the physiological parameters and antioxidant enzymes activities of plant. Unfortunately, there is little research in this area.

Arundo donax L. is a perennial rhizomatous grass (Poaceae family), native to the freshwater regions of Eastern Asia. Because of its high biomass, stronger adaption and unique physiological features whereby it readily absorbs and concentrates toxic chemicals from contaminated soil (Elhawat et al. 2014), A. donax is widely cultivated to yield non-food crop and bio-accumulator, especially via phytoremediation processes (Elhawat et al. 2014). Therefore, there are more and more researches have payed attention to A. donax as trace element bio-accumulator, energy forage and biocar (Srivastava et al. 2010; Elhawat et al. 2014). Although there are numerous data on the physiological and biochemical parameters, photosynthesis and heavy metal pollution of the soil, such as Pb, Cd and Cu et al., it was little know about the effects of TI pollution on plants.

Therefore, the objective of this study was to monitor the responses of chlorophyll fluorescence, photosynthetic parameters and antioxidant enzymes activities of A. donax to different level TI stress. TI uptake and translocation by A. donax were highlighted as well.

Materials and methods
Plant and soil
The tested soil belongs to red clay. Surface soil (0-20 cm) sample and water was collected from the garden of the Guangxi Institute of Botany and the basic physicochemical properties were analyzed (Table I). Tested soil (5 kg) was potted in each plastic tackle and 4 L of water in soil, of which the diameter was 35 cm and the height was 50 cm. The treatments included control (no metal) and five doses
of Thallium (I) Chloride i.e., 0, 0.2, 0.5, 1, 2.5 and 50µg/L Tl.

Table I. Basic physiochemical properties of tested soil and water

<table>
<thead>
<tr>
<th>Item</th>
<th>Physiochemical properties*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td></td>
</tr>
<tr>
<td>PH</td>
<td>6.70</td>
</tr>
<tr>
<td>TN (mg/L)</td>
<td>1.167</td>
</tr>
<tr>
<td>TP (mg/L)</td>
<td>0.802</td>
</tr>
<tr>
<td>TK (g/kg)</td>
<td>0.782</td>
</tr>
<tr>
<td>T-Tl (mg/L)</td>
<td>0.002</td>
</tr>
<tr>
<td>Water</td>
<td></td>
</tr>
<tr>
<td>PH</td>
<td>7.38</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>1.56</td>
</tr>
<tr>
<td>SpC (µS/cm)</td>
<td>235.00</td>
</tr>
<tr>
<td>[Ca2+] (mg/L)</td>
<td>53.00</td>
</tr>
<tr>
<td>T-Tl (µg/L)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

* TN: Total nitrogen; TP: Total phosphorus; TK: Total Kalium; T-Tl: Total Thallium; DO: dissolved oxygen; SpC: specific conductance.

Culture and harvest for plant

During the growing period of A. donax, the height of water was always maintained at 4L of water in soil. After being cultivated for 120 days, chlorophyll fluorescence parameters, photosynthetic gas exchange parameters and antioxidant enzymes activities were determined. After that, the plants were harvested and carefully washed with tap water and deionized water. Leaves, stems and roots were then separated and cut with stainless steel scissor, and dried at 40°C for 48 h for elemental analysis. Total Tl in the plant material was estimated after digestion of oven-dried plants (100mg) following the protocol of Srivastava and D’Souza (Srivastava and D’Souza 2010).

Photosynthetic parameters

Chlorophyll fluorescence parameters and photosynthetic gas exchange parameters were determined by the method described by Lichtenthaler (Genty et al. 1989; Lichtenthaler et al. 2005) using LI-6400XT (Li-Cor, Inc., USA) and portable fluorometer (Monitoring-PAM, Walz, Germany), separately. Photosynthetic rate (Pn), transpiration rate (Tr), intercellular CO₂ concentration (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 controlplants were first light- and dark-adapted for 20 min to obtain F₀ and Fₐ. The Fₘ/and Fₐ values (maximum fluorescence yield of light- and darkadapted leaves, respectively) were calculated with a saturation pulse, and then the maximum photosystem II quantum yield was calculated by the formula [(Fₘ−F₀)/Fₐ=Fₐ/Fₘ]. The effective quantum yield of PSII, Y(II)=(Fₘ−F)/Fₐ, was determined according to Genty (Asithir et al. 2010). All measurements were taken from five plants of each replication during 8:00 to 11:00 a.m.

Antioxidant enzymes activities

The activities of SOD, POD and CAT were assayed by following the protocols of Shah (Shah et al. 2012) with slight modification. Leaves (0.3g) were homogenized in 5 cm3 of ice-cold 50 mM phosphate buffer pH 6.5 (for POD, SOD) and pH 7.5 (CAT). The extracts were centrifuged at 10000 g for 20 min at 0 to 4°C in a Beckmann refrigerated centrifuge, and the supernatants were used for the enzyme activity assays.

Data analysis

All data were statistically analyzed using the SPSS package (Version 18.0). Tl accumulation values are expressed as means ± standard deviation (SD) of the four replicates. ANOVA using was applied to assess significant differences among various treatments. Statistically significant differences were set with P < 0.05, unless otherwise stated.

Results

Accumulation of Tl in A. donax

The accumulation of Tl in the leaves, shoots and roots had a similar trend with the increase of Tl concentration in soil-water, which showed that the amount of Tl accumulation in the grasses firstly increased (from 0 to 2.5µg/L) and then decreased (from 2.5 to 50µg/L) with increasing Tl in soil-water (TABLE II). And the Tl concentration in the leaves, shoots and roots among the treatments was significantly different (TABLE II). For example, at first, the accumulation of Tl in the leaves, shoots and roots of A. donax linearly increased from 0.02, 0.03 and 0.33 to 8.99, 2.96 and 14.62mg and then decreased to 7.00, 2.37 and 11.95 mg, respectively. In addition, the Tl concentration in the grass roots was significantly higher than that in the grass leaves, shoots (TABLE II).

Table II. Concentrations of Tl in dry giant reed plants after 4-month cultivation (mg/kg)

<table>
<thead>
<tr>
<th>Treatments (µg/L)</th>
<th>Content of Tl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves (mg/kg)ᵇ</td>
</tr>
<tr>
<td>0</td>
<td>0.02ᵇ</td>
</tr>
<tr>
<td>0.2</td>
<td>2.26ᵇ</td>
</tr>
<tr>
<td>0.5</td>
<td>3.50ᵇ</td>
</tr>
<tr>
<td>1</td>
<td>4.97ᵇ</td>
</tr>
<tr>
<td>2.5</td>
<td>8.99ᵇ</td>
</tr>
<tr>
<td>50</td>
<td>7.00ᵇ</td>
</tr>
</tbody>
</table>

ᵇ Data with a single star (*) indicate a significant difference at P < 0.05 among them, with double stars (**) indicate a significant difference at P < 0.001 among them.

Chlorophyll fluorescence parameters

There were no differences on relative chlorophyll content, primary photosynthetic efficiency of PS II (Fv/Fm) and Yield (Fig.1) under the lower level Tl treatment (0.2-2.5µg/L). However, higher level Tl treatment (50µg/L) significantly inhibited Chlorophyll synthesis, and thus reduced Fv/Fm, Fv/F0 and Yield (Fig.1; P < 0.05). In addition, compared with control, median level Tl treatment (0.5 and 2.5µg/L) also reduced Fv/Fm (Fig.1c; P < 0.05).
Chlorophyll fluorescence parameters of *A. donax* in the five concentrations of Th treatments. Different lowercase letters on the top of the bars denote significant differences ($P < 0.05$) among different Th treatments.

**Gas Exchange Parameters**

In addition to 2.5µg/L Th treatment, Th-induced stress drastically decreased the photosynthetic rate (Pn) in the leaves of *A. donax* (Fig.2a). And with the increase of Th concentration the changing of Pn, Tr and Gs showed a similar trend (Fig. 2). For example, at first, the Pn decreased with increasing Th (from 0 to 0.5µg/L) and then increased with increasing Th (from 0.5 to 2.5µg/L), but again decreased with higher Th lever (50µg/L) treatment (Fig. 2a). While, intercellular CO$_2$ concentration (Ci) showed a contrary trend with Pn. Th-induced stress was not effected (0.2 and 1µg/L) or increased (0.5 and 5µg/L) intercellular CO$_2$ concentration, but decreased under 2.5µg/L Th treatment (Fig. 2c).

**Antioxidant enzymes activities**

In plants exposed to Th, significant increase in POD in comparison to control, but significant decrease in SOD in comparison to control (Fig.3). And CAT showed a higher activity under higher Th (from 1 to 50µg/L) but a lower activity under lower Th (0.5µg/L) (Fig. 3a).

**Discussion**

Previous studies indicated that the distribution of heavy metals (Cu, Zn and Cd *et al.*; Srivastava and D’Souza 2010; Nasso *et al.* 2013) in *A. donax* was higher in roots than that in stems and leaves. This was confirmed in present study by Th concentration in the grass roots being significantly higher than that in the grass leaves and shoots. One possible reason was that the root system of *A. donax* has a clear rejection to Th during conveying nutrition from roots to leaves. As a result, it can be used as a barrier to make it difficult for Th to migrate to the ground, and to make the ground part from its harm. Recent study also showed that *A. donax* is able to store nutrients during the growing period and partially release them to support rapid stem growth in the spring (Asthir *et al.* 2002). Hence, root system of *A. donax* was able to not only store nutrients but also hold back Th during conveying nutrition from roots to aboveground parts. Despite its higher interception function in root system of *A. donax* to nutrition and Th, the amount of Th accumulation in the grasses increased with increasing Th (from 0 to 2.5µg/L), and was still higher under high level Th (50µg/L) than lower level Th (< 2.5µg/L), suggesting that *A. donax* was a tolerant plant species to Th.

In general, antioxidant enzymes activities are common mechanisms in plants to regulate the ROS produced by these plants as a result of metabolic processes (Liu *et al.* 2014). Our results showed that SOD decreased under Th stress, suggesting that Th pollution (four months) attenuated the conversion ability of toxic to H$_2$O$_2$ since SOD is the key enzyme in the active oxygen scavenger system that dismutates O$_2$ into H$_2$O$_2$ (Han *et al.* 2010). Previous studies showed that long time (more than 20-day) metal stress always decreased SOD (Liu *et al.* 2014) by inhibition of enzyme syntheses and change in the assemblage of
enzyme subunits (Deng et al. 2013). This was confirmed in present study. While, POD and CAT increased under high lever Tl stress, showing H2O2 converts increased under Tl stress since CAT converts H2O2 into water and molecular oxygen (O2) and POD decomposes H2O2 by oxidation of substrates (Nakano et al.1981). It seems to contradict with previous studies in which SOD, CAT and POD of plant always showed a similar trend in a short time (Liu et al. 2014). One possible reason may be that in the development of plant, the activities of POD and CAT in leaves of plant are constantly changing and are higher in old tissues than that in young tissues since the peroxidase can make certain carbohydrates, contained in tissue, into lignin, and increase its degree of lignification (Harskamp et al. 2010). At the same time, the tested time of the antioxidant enzymes activities was carried out in November, thus leaves of A. donax were most of aging stage. Another reason may be that Tl+ can interfere with Na+/K+ ATPase, pyruvate kinase and membrane phospholipid, thus damage DNA by inducing oxidative stress (Siegel and Siegel 1976) and alter membrane physical properties (Küpper et al. 2005). As a result, a lot of Tl+ get into the cytoplasm leading to the increase of ROS in cell cytoplasm and cause the decrease of SOD activity since Tl+ has a high affinity with the amino-group, imino group and sulfydryl of protein and other biological macromolecules (Nakano et al.1981).

Chlorophyll fluorescence and photosynthetic parameters are important to study physiological responses of plants against metal-induced stress (Balakhnina et al. 2005). Especially, under high level Tl stress (50µg/L), the structure of PS II may be destroyed and resulted in a decrease in relative chlorophyll content (SPAD), Fv/Fm, Fv/F0 and Y(II). One possible reason may be that reduce of these chlorophyll fluorescence in this study could be the result of functional disorder of antenna complexes that raised F0 and thereby reduced Fv/Fm, Fv/F0 and consequently reduced the plant photosynthesis (Wang et al. 2014). At the same time, once the structure of PS II being destroyed, photosynthetic activity would reduce and the light energy absorbed by the leaves could not be converted into chemical energy, which could inhibit the initial reaction of leaf photosynthesis (Liu et al. 2010). In this study, Tl application mainly negatively influenced various photosynthetic parameters like Pn, Tr and Gs and SOD activity. While intercellular CO2 concentration (Ci) showed a contrary trend with Pn due to the effect of nonstomatal factors. At the same time, POD and CAT increased under high lever Tl stress, showing H2O2 converts increased after 4-month growing of A. donax.

Acknowledgment

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