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 (Tl) 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 Tl separately set 0 (CK), 0.2, 0.5, 1, 2.5 and 50μg/L. The amount of Tl accumulation in leaves, stems and roots of *A. donax* increased with increasing Tl (from 0 to 2.5μg/L), and was still higher under high level Tl (50μg/L) than lower level Tl (< 2.5μg/L). However, high level Tl stress (50μg/L) was significantly inhibited chlorophyll synthesis, and thus reduced the primary photochemical efficiency of PSII (Fv/Fm), potential activity of PSII (Fv/Fo), apparent quantum (Yield). Meanwhile, Tl application mainly negatively influenced various photosynthetic parameters like Pn, Tr and Gs and SOD activity. Nevertheless, intercellular CO_2 concentration (Ci) showed a contrary trend with Pn due to the effect of nonstomatal factors, and POD and CAT increased under high lever Tl stress, showing H_2O_2 converts increased after 4-month growing of *A. donax.* This study suggests that *A. donax* was a tolerant plant species to Tl may be mainly through induced antioxidant machinery.

Keywords Arundo donax,antioxidant enzymes, chlorophyll fluorescence, Thallium

INTRODUCTION

Thallium (Tl) 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, Tl has attracted increasing concerns (Gomez-Gonzaleza *et al*. 2015). Extensive Tl-containing ore mining, smelting, and Tl-containing fossil fuel burning are the major sources of anthropogenic dispersion of Tl in the environment (Siegel and Siegel *et al*. 1976). Although there has been some progress in Tl environmental geochemistry and ecotoxicology, the toxic mechanism of Tl is not entirely clear.

As Tl^+ 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, Tl^+ 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. Tl pollution can often lead to the accumulation of Tl in the chloroplasts of plants, which would result in the physiological effects of photosynthesis, antioxidant enzymes activities since Tl^+ 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 Tl 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 *et al*. (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 Tl 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 Tl stress. Tl 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 Institue of Botany and the basic physiochemical properties were analyzed (TABLE I). Tested soil (5kg) was potted in each plastic tackle and 4L 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

Item	**Physiochemical properties**^{a}				
Soil	**PH**	**TN (mg/L)**	**TP (mg/L)**	**TK （g/kg）**	**T-Tl (mg/L)**
	6.70	1.167	0.802	0.782	0.002
Water	**PH**	**DO (mg/L)**	**SpC (μs/cm)**	**$[Ca^{2+}]$ （mg/L）**	**T-Tl (μg/L)**
	7.38	1.56	235.00	53.00	0.005

[a] 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 CO_2 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 CO_2 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 F and F_0. The Fm’and Fm 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−Fo)/Fm=Fv/Fm]. The effective quantum yield of PSII, Y(II)=(Fm−F)/Fm, was determined according to Genty (Asthir *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)

Treatments (μg/L)	**Content of Tl**		
	Leaves* *(mg/kg)^{b}	***Shoots* *(mg/kg)***^{b}	***Roots* *(mg/kg)***^{b}
0	0.02^{**}	0.03^{**}	0.33^{**}
0.2	2.26^{**}	1.08^{**}	5.03^{*}
0.5	3.50^{**}	1.59^{**}	6.94^{*}
1	4.97^{**}	2.12^{*}	8.73^{**}
2.5	8.99^{**}	2.96^{**}	14.62^{**}
50	7.00^{**}	2.37^{*}	11.95^{**}

[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 photochemical efficiency of PS II (Fv/Fm) and Yield (Fig.1) under the lower lever 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, medial lever Tl treatment (0.5 and 2.5μg/L) also reduced Fv/Fm (Fig.1c; $P < 0.05$).

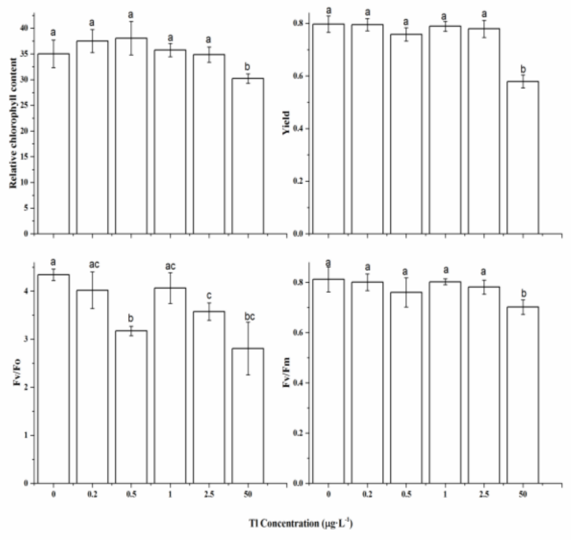

Fig. 1 Chlorophyll fluorescence parameters of *A. donax* in the five concentrations of Tl treatments. Different lowercase letters on the top of the bars denote significant differences ($P < 0.05$) among different Tl treatments.

Gas Exchange Parameters

In addition to 2.5μg/L Tl treatment, Tl-induced stress drastically decreased the photosynthetic rate (Pn) in the leaves of *A. donax* (Fig.2a). And with the increase of Tl concentration the changing of Pn, Tr and Gs showed a similar trend (Fig. 2). For example, at first, the Pn decreased with increasing Tl (from 0 to 0.5μg/L) and then increased with increasing Tl (from 0.5 to 2.5μg/L), but again decreased with higher Tl lever (50μg/L) treatment (Fig. 2a). While, intercellular CO_2 concentration (Ci) showed a contrary trend with Pn. Tl-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 Tl treatment (Fig. 2c).

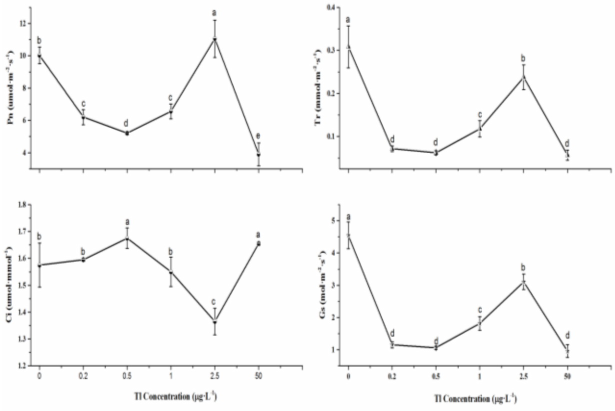

Figure 2. Gas exchange parameters of *A. donax* under different level Tl conditions. Different lowercase letters on the top of the bars denote significant differences *(P* < 0.05) among different Tl treatments.

Antioxidant enzymes activities

In plants exposed to Tl, 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 Tl (from 1 to 50μg/L) but a lower activity under lower Tl (0.5μg/L) (Fig. 3a).

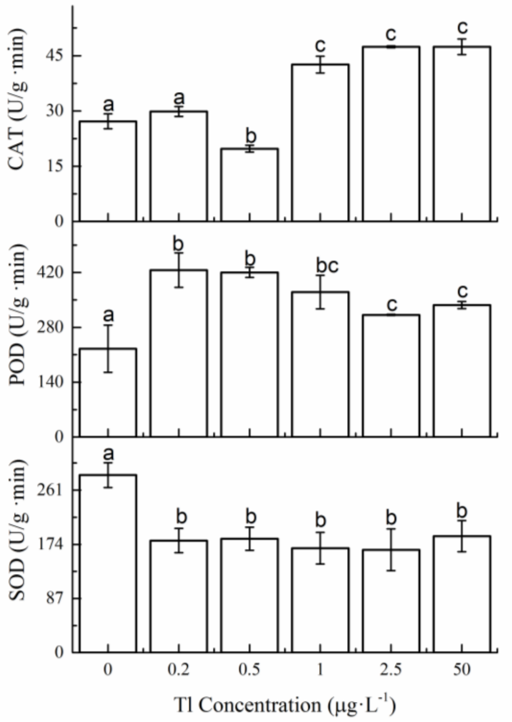

Figure 3. Effect of Tl stress on SOD, COD and CAT activity in leaf of *A. donax*. Different lower case letters on the top of the bars denote significant differences ($P < 0.05$) among different Tl treatments.

DISCUSSION

Previous studies indicated that the distribution of heavy metals (Cu, Zn and Cd *et al.*) in *A. donax* was higher in roots than that in stems and leaves (Srivastava and D'Souza 2010; Nasso *et al.* 2013). This was confirmed in present study by Tl 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 Tl during conveying nutrition from roots to leaves. As a result, it can be used as a barrier to make it difficult for Tl 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 Tl during conveying nutrition from roots to aboveground parts. Despite its higher interception function in root system of *A. donax* to nutrition and Tl, the amount of Tl accumulation in the grasses increased with increasing Tl (from 0 to 2.5μg/L), and was still higher under high level Tl (50μg/L) than lower level Tl (< 2.5μg/L), suggesting that *A. donax* was a tolerant plant species to Tl.

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 Tl stress, suggesting that Tl pollution (four months) attenuated the conversion ability of toxic to H_2O_2 since SOD is the key enzyme in the active oxygen scavenger system that dismutates $O_2^{\cdot -}$ into H_2O_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 conformed in present study. While, POD and CAT increased under high lever Tl stress, showing H_2O_2 converts increased under Tl stress since CAT converts H_2O_2 into water and molecular oxygen (O_2) and POD decomposes H_2O_2 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 *et al.* 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 (except to 2.5μg/L Tl treatment), Tr and Gs. The possible reason may be that the improvement of Pn mainly profited from the increase of Tr and Gs (Liu *et al.* 2010). While, with the decrease of Pn, Ci increased, suggesting the change of photosynthetic capacity of plant is caused by nonstomatal factors. The possible reason may be the decrease of photosynthetic activity of mesophyll cells (Liu *et al.* 2010).

In conclusion, despite its higher interception function in root system of *A. donax* to nutrition and Tl, the amount of Tl accumulation in the grasses increased with increasing Tl (from 0 to 2.5μg/L), and was still higher under high level Tl (50μg/L) than lower level Tl (< 2.5μg/L). The accumulation of Tl in leaves, stems and roots increased with the increase of Tl concentration in soil-water. And high level Tl stress (50μg/L) was significantly inhibited chlorophyll synthesis, and thus reduced the primary photochemical efficiency of PSII (Fv/Fm), potential activity of PSII (Fv/F0), apparent quantum (Yield). Tl application mainly negatively influenced various photosynthetic parameters like Pn, Tr and Gs and SOD activity. While intercellular CO_2 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 H_2O_2 converts increased after 4-month growing of *A. donax*.

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References

Alshaal T., Domokos-Szabolcsy E., Marton L. J., Katai, Balogh P., Elhawat N., El-Ramady H., Fari M., "Phytoremediation of bauxite-derived red mud by giant reed. Environ," Chem. Lett, vol. 11, pp. 295-302, September 2013.

Asthir B., Duffus C.M., Smith R., Spoor W., "Cell and Molecular Biology, Biochemistry and Molecular Physiology. Diamine oxidase is involved in H_2O_2 production in the chalazal cells during barley grain filling," J. Exp. Bot, vol. 53, pp. 677-682, April 2002.

Asthir B., Koundal A., Bains N.S., Mann S.K., "Stimulation of antioxidative enzymes and polyamines during stripe rust disease of wheat," Biol. Plantarum, vol. 54, pp. 329-333, June 2010.

Balakhnina T.I., Kosobryukhov A.A., Ivanov A.A., Kreslavskii V.D., "The effect of cadmium on CO_2 exchange, variable fluorescence of chlorophyll, and the level of antioxidant enzymes in pea leaves," Russ. J. Plant Physiol.-Engl. Tr, vol. 52, pp. 15-20, January 2005.

Belowitz R., Donnell M.J.O., "Ion-selective microelectrode measurements of Tl(+) and K(+) transport by the gut and associated epithelia in Chironomus riparius," Aquat. Toxicol, vol.138-139, pp. 70-80, August 2013.

Deng H.M., Chen Y.H., Liu T., Wu C.Q., Qiu L., Wu GM., Zeng D.M., "Environmental Chemistry 32, 9 (2013) Study on the translocation and accumulation of thallium in soil-plant system," Environmental Chemistry, vol. 32, pp. 1749-1757, September 2013.

Elhawat N., Alshaal T., Domokos-SzabolcsyÉ., El-Ramady H., Márton L., CzakóM., Kátai J., Balogh P., Sztrik A., Molnár M., Popp J., Fári M.G., "Phytoaccumulation potentials of two biotechnologically propagated ecotypes of Arundo donax in copper-contaminated synthetic wastewater," Environ Sci. Pollut. Res , vol. 21, pp. 7773-7780, June 2014.

Genty B., Briantais J.M., Baker N.R., "The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence," Biochim. Biophys. Acta, vol. 990, pp. 87-92, January 1989.

Gomez-Gonzaleza M.A., Garcia-Guineaa J., Labor F., Garrido F., "Thallium occurrence and partitioning in soils and sediments affected by mining activities in Madrid province (Spain)," Sci. Total. Environ, vol. 536, pp. 268-278, December 2015.

Han Z.P., Yang Z.H., Wu X., Zhang H., “Effects of Lead Stress on the Antioxidant Enzymes Activities in Arundo Donax Linn,” Journal of Nuclear Agriculture Sciences, vol. 24, pp. 846-850, August 2010.

Harskamp J. G., Donnell M. J., Berkelaar E., “Determining the fluxes of Tl^+ and K^+ at the root surface of wheat and canola using Tl(I) and K ion-selective microelectrodes,” Plant. Soil, vol. 335, pp. 299-310, October 2010.

Küpper H., Kroneck P.M.H., “Heavy metal uptake by plants and cyanobacteria.” Metal Ions in Biological Systems, vol. 44, pp. 97-144, February 2005.

Lichtenthaler H.K., Langsdorf G., Lenk S., Buschamann C., “Chlorophyll fluorescence imaging of photosynthetic activity with the flash-lamp fluorescence imaging system,” Photosynthetica, vol. 43, pp. 355-369, September 2005.

Liu H.G., Hu C.X., Sun X.H., Tan Q.L., Nie Z.J., Hu X.M., “Interactive effects of molybdenum and phosphorus fertilizers on photosynthetic characteristics of seedlings and grain yield of Brassica napus,”. Plant. Soil, vol. 326, pp. 345-353, January 2010.

Liu N, Lin Z, Guan L， Gaughan G, Lin G, “Antioxidant enzymes regulate reactive oxygen species during pod elongation in Pisum sativum and Brassica chinensis,” Plos One, vol. 9, pp. e87588, February 2014.

Liu T.， Deng H.M., Wang Y.L., Chen Y.H.， Chen M.C. ， Wu G.M.， Zeng D.M., Du G.Y.， Gu Y.S.， Xu X.C., “Growth Inhibition of Thallium(I)- nitrate on Duckweed (Lemna gibba L.) with Relation to Its Oxidative Stress,” Asian Journal of Ecotoxicology, vol. 9, pp. 1112-1117, December 2014.

Nakano Y., Asada K., “Hydrogen Peroxide is Scavenged by Ascorbate-specific Peroxidase in Spinach Chloroplasts,” Plant Cell Physiol,1981, 22(5):867-880, vol. 22, pp. 867-880, October 1981.

Nasso N.D., Roncucci N., Bonari E., “Seasonal Dynamics of Aboveground and Belowground Biomass and Nutrient Accumulation and Remobilization in Giant Reed (Arundo donax L.): A Three-Year Study on Marginal Land,” Bioenerg. Res, vol. 6, pp. 1-12, June 2013.

Renkema H., Koopmans A., Hale B., Berkelaar E., “Thallium and potassium uptake kinetics and competition differ between durum wheat and canola,” Environ. Sci. Pollut. Res, vol. 22, pp. 2166-2174, August 2015.

Shah K， Nahakpam S, “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. Physiol. and Bioch, vol. 57, pp. 106-113, Augus 2012.

Siegel B.Z., Siegel S.M., “Effect of potassium on thallium toxicity in cucumber seedlings: further evidence for potassium-thallium ion antagonism,” Bioinorg. Chem, vol. 6, pp. 341-345, December 1976.

Srivastava S., D'Souza S.F., Royle, “Effect of variable sulfur supply on arsenic tolerance and antioxidant responses in Hydrilla verticillata (L.f.) Royle,” Ecotoxicol. Environ. Saf, vol. 73, pp. 1314-1322, September 2010.

Wang L., Xu Y.M., Sun Y.B., Liang X.F., Lin D.S., “Identification of pakchoi cultivars with low cadmium accumulation and soil factors that affect their cadmium uptake and translocation,” Front. Environ. Sci. Eng, vol. 8, pp. 877-887, December 2014.