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Biotransformation and metabolic response of cyanide in weeping willows

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Abstract

Biotransformation and metabolic responses of plants to cyanide were investigated using pre-rooted plants of weeping willows (*Salix babylonica* L.) grown hydroponically in growth chambers and treated with potassium cyanide. Various physiological parameters of the plants were monitored to determine toxicity from exogenous cyanide exposure. Cyanide doses used in this study showed growth-promoting effects on plants, exhibiting higher measured values of transpiration rates, chlorophyll contents and soluble protein contents compared with the non-treated control plants. Superoxide dismutases (SOD), catalase (CAT) and peroxidase (POD) activities in leaves showed a slight change to cyanide application in most treatments. Of all selected parameters, soluble proteins of plants were the most sensitive indicator to cyanide application. Almost all applied cyanide was removed from the hydroponic solution in the presence of plants in all treatment groups. Small amounts of cyanide were detected in the plant tissues. Recovery of cyanide in different compartments of plants varied significantly, root being the dominant sink for cyanide accumulation. Mass balance studies showed that >97% of the applied cyanide was metabolized during transport through weeping willows and the metabolic rates of cyanide by plants were linearly increased with increasing of cyanide applied in the growth media. Results from this study indicated that neither visible toxic symptom nor metabolic lesion was observed for the plants after 192 h of exposure, largely due to the well-established detoxification systems in willows. These findings suggest that cyanide has a beneficial role in plants and phytoremediation is a desirable solution of treating environmental sites contaminated with cyanide.

Keywords: Cyanide; Enzyme; Oxidative stress; Phytoremediation; Removal; Willows

1. Introduction

Cyanide naturally occurs in the environments, for example, by synthesis from cyanogenic glycosides in plants [1]. However, more than 100,000 tons of cyanide enters the environment annually due to anthropogenic activities [2]. A couple of disastrous events with cyanide compounds, such as the accidental release of methyl isocyanate in the Union Carbide Manufactory in Bhopal December 1984 [3], the cyanide spill at Baia Mare, Romania 2000 [4], repeated cyanide spills from the Ashanti gold fields in Ghana and accidents in PR China were reported [3,5].

Cyanide functions as a nitrogen source or potent toxicant to plants, highly depending on its concentrations. Although cyanide is involved in several common plant biochemical pathways [6], unacceptable high concentrations of cyanide are highly toxic to plants [7–9]. Levels of $\geq$5 mg KCN L^{-1} in hydroponic solution were toxic to basket willows (*Salix viminalis*), levels $\geq$20 mg KCN L^{-1} were immediately lethal [9]. EC_{50} values for weeping willows (*Salix babylonica* L.) were estimated to be between 3.27 and 8.23 mg CN L^{-1} using normalized transpiration rate as a sensitive end point for toxicity determination, depending on the duration of exposure period [8]. The toxicity of cyanide is generally ascribed to the formation of complexes with metal ions that are present as enzyme cofactors. For example, cyanide occurs with ferric ion in cytochromes, thereby inhibiting respiration and hence, oxidative phosphorylation [10]. However, the assimilation of cyanide in plants has been reported by Miller and Conn [11]. Cyanide is efficiently converted to the final metabolite asparagine in the presence of the enzymes beta-cyanoalanine synthase and beta-cyanoalanine hydrolase [12,13]. All vascular plants share a common feature in metabolizing free cyanide and a series of studies was carried out to uncover

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the kinetics of the detoxification system of the plants and the relationship with uptake and toxicity [5–9,11,14–16]. Twenty-eight Chinese plant species from 23 families were tested for their cyanide removal capacity in a closed bottle test [5], and all species were able to metabolize cyanide but with different rates. The fastest cyanide removal was by Chinese elder, *Sambucus chinensis*, with a removal capacity of 8.8 mg CN kg^{-1} h^{-1}. The weeping willow, *S. babylonica* L. had a removal capacity of 6.08 mg CN kg^{-1} h^{-1}. Ebbs et al. found that free cyanide is rapidly taken up and is metabolized in willows (*Salix eriocephala* L. var. Michaux), using labeled free cyanide [6]. Uptake, metabolism, accumulation and toxicity of cyanide were also determined on willow trees [7,9]. Additional data on the Michaelis–Menten kinetics of cyanide removal by 12 plant species out of 9 families were also obtained [14] and the values of v_{max} and K_M were between 6.68 and 21.91 mg CN kg^{-1} h^{-1} and 0.90 to 3.15 mg CN L^{-1}, respectively.

Unfavorable environmental conditions often lead to an increased formation of reactive oxygen species (ROS) in plants [17], e.g., O^{2-} and H_2O_2 are generated as intermediates of a number of metabolic reactions in cellular organelles of different plants, leading to damage of DNA, proteins and pigments as well as initiating lipid peroxidation [18]. Consequently, the antioxidant enzymatic systems of plant have been proposed to play an important role in regulating plant stress tolerance [19]. Superoxide dismutases (SOD) are metalloproteins responsible for converting superoxide radicals (O^{2-}) to hydrogen peroxide (H_2O_2) and its subsequent reduction to H_2O [18]. Catalases (CAT) and peroxidases (POD) catalytically scavenge H_2O_2 formed in plant cells and provide the necessary defenses [20]. Little information is available concerning the metabolic responses of vascular plants to cyanide exposure. The present study investigated the uptake and bioaccumulation potential of cyanide in weeping willows, a native Chinese species (*S. babylonica* L.) grown in hydroponic solution and the effects of cyanide on metabolic activities of plants, with the objective to provide quantitative information for risk assessment whether cyanide phytoremediation is ecologically safe.

2. Materials and mthods

2.1. Trees specimens and exposure regimes

Weeping willows (*S. babylonica* L.) were taken from those grown on the campus of Hunan Agricultural University, PR China. Preparation of willow cuttings was performed as described by Yu et al. [17] previously. The flasks with plant cuttings and the modified ISO 8692 standard nutrient solution [8] were housed in a climate control chamber kept at a constant temperature of 24.0 ± 1 °C under natural sunlight (light:dark cycle 14:10 h). After a 48-h period of pre-adaptation, the nutrient solution was replaced by spiked solution, except for the controls. Cyanide used was in the form of potassium cyanide KCN of analytical grade with ≥95% purity. It should be noted that 1 mg KCN equals to 0.40 mg CN. Several concentrations (0.30, 0.59, 1.19, 2.37 and 4.74 mg CN L^{-1}) were prepared by adding the required aliquots of 0.948 g CN L^{-1} stock solution of KCN to the nutrient solution. For each treatment concentration, nine replicates were conducted. Two sets of controls were made: one control was with cyanide, but without plant cuttings to quantify the effects of loss during handling, volatilization, hydrolysis and/or degradation by microorganism; the other was with trees in the nutrient solution without addition of cyanide to quantify the transpiration rate of the non-exposed control trees. The weight loss of the plant-flask system was expressed as the transpiration rate [21].

2.2. Chlorophyll measurement

The chlorophyll content in leaves was determined spectrophotometrically at the end of the experiments (192 h). 0.5 g of leaves (fresh weight) was placed in 25 mL flasks. Then, 80% acetone was filled to the mark of 25 mL. Three separate flasks were conducted for each treatment group. All flasks were placed in the dark for 24 h. During this period, flasks were shaken twice. The absorption of light at 645 and 663 nm was measured in a cell with an optical path of 10 mm against 80% acetone as a blank. The amount of chlorophyll a and chlorophyll b in plant leaves was calculated by the Maclachalam and Zalik's equation [22].

2.3. Enzyme activity measurement

The activities of three antioxidant enzymes SOD, CAT and POD were measured in fresh leaves at the end of the experiment. 0.3 g of leaves (fresh weight) was precisely weighted and placed in a triturator. 1.4 mL of phosphate buffer solution (pH 7.8, containing NaH_2PO_4, Na_2HPO_4, PVPP, EDTA and mercapto-ethanol) were added before trituration. Trituration was performed in an ice-bath and then centrifuged at 8,000 rpm for 15 min, the supernatant was collected and stored at 4 °C and employed in the enzyme assays. Each enzyme was measured independently. SOD, POD and CAT activities in leaf cells were determined spectrophotometrically as described by Yu et al. [17] previously.

2.4. Soluble protein measurement

The soluble protein content was determined spectrophotometrically in fresh leaves from the top shoot as described by Jin and Ding [23]. At the end of the experiments (192 h), 0.5 g of tissue materials (fresh weight) was precisely weighted and placed in a triturator. 2.5 mL of 65 mM phosphate buffer solution (pH 7.8) containing 0.4% mercapto-ethanol (v/v) was added before trituration. Triturating was performed in an ice-bath and then centrifuged at 12,000 × *g* for 15 min. The supernatant was stored at 4 °C before analyzing the soluble protein in leaves. 0.1 mL aliquot of the samples was pippetted into a vessel and 5 mL Coomassie Brilliant Blue G-250 solution (Sigma–Aldrich Inc., St. Louis, Missouri) were added. After mixing, the vessel was left standing for 2 min. The absorption of light at 595 nm was measured spectrophotometrically against water as reference. Albumin bovine V solution from bovine serum (Sigma–Aldrich Inc.) was used as a standard.

2.5. Chemical analysis

2.5.1. Cyanide in solution

A stock solution concentration at 0.948 g CN L^{-1} of potassium cyanide was prepared. The concentration of cyanide in the aqueous solution was determined photometrically by a standard method (State Environmental Protection Administration of China). The 1–10 mL solution of solution samples were pipetted into a 25 mL colorimetric cylinder (depending on the concentraions of cyanide in solution), and 1% NaOH was added to the mark of 10 mL. Then 5.0 mL of a buffer solution with potassium dihydrogen phosphate and sodium phosphate were added. Quickly 0.2 mL of 1% (m/v) chloramine-T solution were introduced. The vessel was sealed with a stopper and left standing for 3–5 min. The 5 mL of the color reagent consisting of isonicotinic acid and 3-methyl-1-phenyl-5-pyrazolone were then added. The content was diluted with deionized water to the mark (25 mL) and mixed thoroughly. Finally, the colorimetric cylinders were all kept in a water bath at a temperature of 32 °C for 40 min. The absorption of light at 638 nm was measured in a cell of optical path length of 10 mm against a water as reference. All chemicals used were >99.5% purity, except potassium cyanide and nicotinic acid, which were technical grade (92–95% purity); but the stock solution and the standard solution of KCN used in this test were calibrated by a standard solution of $AgNO_3$, which was also calibrated by a standard solution of NaCl (standard method from SEPA, PR China). The detection limit of this method was determined from blank +3 standard deviations of 10 replicates to be 0.004 mg CN L^{-1} with 10 mL sample volume.

2.5.2. Total cyanide in plant materials

Total cyanide is the sum of easy liberated cyanide and complexed cyanide. The total cyanide in plant materials was analyzed according to the methd by Yu et al. [24]. Ten milliters of 1% NaOH was added into the absorption vessel of the distillation unit. Fresh plant biomass (2.5–15 g FW, depending on the harvested weight of plant materials) cut into samll pieces were placed in a 500 mL round bottom flask, and then 200 mL of distilled water was added. Then 10 mL of sodium ethylenediamine tetraacetate with a concentration of 10% (m/v) and 10 mL of phosphoric acid (≥85% purity) were added before heating and mixing. Approximately 100 mL distilled solution containing cyanide from plant materials were collected, quantitatively transferred to a 100 mL volumetric flask and made up to the volume with deionized water. The solution was stored at below 6 °C until the concentration of cyanide was determined. The samples were all analyzed within a maximum hold time of 4 h. The remaining procedure was identical to those described earliar.

2.6. Statistical methods

The students *t*-test (one-tailed) and the Pearson's product-moment correlation and regression were conducted. The significance of the correlations was judged using tabled values for the critical r (degree of freedom *n*-2, significance level α was 0.01 or 0.05) from Sachs [25]. The partial correlation was used to determine whether a relationship between two variables was due to a common correlation to a third variable, with the equation

$$r_{xyz} = \frac{r_{xy} - r_{xz} \times r_{yz}}{\sqrt{(1 - r_{xz}^2)(1 - r_{yz}^2)}}$$

where $r_{xy\,z}$ is the partial correlation coefficient between variables x and y under the assumption of a constant variable z, and r_{xy} is the bivariate Pearson correlation coefficient between variables x, y, etc.

3. Results

3.1. Effects of cyanide on the transpiration rate of weeping willows

Transpiration rate of weeping willows grown in hydroponic solution was significantly affected by cyanide after 192 h of exposure (Table 1). The transpiration rate of plants is coupled to the photosynthesis, and an inhibition of transpiration is a reliable and quick measure of toxic effects [21]. A marked increasing trend in the transpiration rate for treated plants was apparent over the increase of cyanide concentrations. Transpiration rate in all treatments was higher than that of the non-treated control plants. Sixteen percent increase in transpiration rate was observed for the weeping willows exposed to 1.19 mg CN L^{-1}, but the difference is insignificant compared with the controls ($p > 0.05$). Significant increase was found for the treatments exposed to ≥2.37 mg CN L^{-1} ($p < 0.05$). Visible toxic symptom, e.g., chlorosis of leaves, was not observed in all treatments during the entire period of exposure. The results implied that cyanide doses used in this study probably stimulated plant growth. Similar results were reported by Yu et al. [8] in which no chlorosis of leaves and a small reduction in normalized relative transpiration was observed for weeping willows grown in hydroponic solution spiked with cyanide ≒0.93 mg CN L^{-1}.

3.2. Effects of cyanide on the content of chlorophylls in plant leaves

Table 1 also shows the measured chlorophyll a and chlorophyll b contents of leaves under different cyanide treatments after 192 h of exposure. Chlorophyll contents in leaves varied with different treatments of cyanide to the willows. All chlorophyll contents of the treated plants were higher than that of the non-treated control plants. Concentrations of both chlorophyll a and b in leaves increased as cyanide application increased from 0.30 to 1.19 mg CN L^{-1}, and then chrolophyll concents were inversely proportional to the increase of cyanide concentrations. The highest contents of chlorophyll a and b were detected to be 0.36 and 0.38 mg g^{-1} FW for the willows exposed to 1.19 mg CN L^{-1}, respectively.

Table 1
Effects of various cyanide treatments on transpiration rate, soluble protein, chlorophyll contents and activities of superoxide dismutases (SOD), peroxidase (CAT) and catalase (POD)

Characteristic	Cyanide concentrations (mg CN L^{-1})					
	0.00	0.30	0.59	1.19	2.37	4.74
Transpiration rate (g d^{-1})	3.28 (1.247)	3.47 (1.223)	3.49 (1.011)	3.82 (1.140)	4.04[a] (1.023)	4.11[a] (0.885)
Chlorophyll a (mg g^{-1} FW)	0.28 (0.022)	0.32 (0.025)	0.32 (0.019)	0.36[a] (0.068)	0.32 (0.093)	0.29 (0.035)
Chlorophyll b (mg g^{-1} FW)	0.28 (0.019)	0.32 (0.031)	0.36[a] (0.022)	0.38[a] (0.088)	0.34 (0.094)	0.28 (0.069)
Soluble protein (mg g^{-1} FW)	4.82 (1.715)	5.18 (0.548)	5.19 (0.596)	5.19 (0.719)	5.44 (1.194)	5.71[a] (1.290)
Superoxide dismutases (U g^{-1} FW)	250.22 (13.079)	250.61 (47.008)	248.46 (11.946)	243.95 (7.019)	241.60 (5.951)	234.93 (23.448)
Peroxidase (U g^{-1} FW)	39.04 (5.261)	37.18 (4.259)	36.40 (0.659)	32.24[a] (2.108)	32.20[a] (3.523)	28.26[a] (2.829)
Catalase (U g^{-1} FW)	195.91 (47.869)	217.51 (32.884)	185.43 (7.493)	175.42 (44.518)	171.12 (41.625)	171.89 (44.123)

Values are the mean of three replicates for both the treated and non-treated control plants, except the transpiration rate (nine replicates for the treated plants and six for the non-treated plants), numeric values in brackets represent standard deviation, FW = fresh weight.
[a] Significantly different to the controls on 95% significance level (one-tailed *t*-test).

3.3. Effects of cyanide on the content of soluble proteins in plant leaves

Soluble protein concentrations in leaves of weeping willows were strongly affected by the presence of cyanide (Table 1). Measured soluble protein contents in leaves of the treated plants were higher than that of the non-treated control plants. Cyanide application had a positive influence on the soluble proteins in leaves. No significant difference in the content of soluble protein ($p > 0.05$) was observed for the plants exposed to $\leq$2.37 mg CN L^{-1}, whereas significant increase in soluble proteins was detected for the plants exposed to 4.74 mg CN L^{-1} ($p < 0.05$).

3.4. Effects of cyanide on the activities of antioxidant enzymes in plant leaves

Activities of SOD, CAT and POD in the leaf cells of weeping willows were also measured at the end of cyanide exposure (Table 1). An observable declining trend in the SOD activity for treated plants was apparent over the increase of cyanide concentrations, but there is no significant difference between the treated plants and the controls ($p > 0.05$). At low cyanide concentration of 0.30 mg CN L^{-1}, activity of SOD was similar to the level of the controls. With increasing cyanide addition, SOD activity gradually decreased with a maximum reduction of 6.11% for the plants exposed to 4.74 mg CN L^{-1}. Such a slight difference in the SOD activity between the treated and the non-treated plants was more likely due to differences in individual tree specimens used.

Activity of CAT in leaves varied with the doses of cyanide (Table 1). At low cyanide concentration of 0.30 mg CN L^{-1}, CAT activities were higher than the controls, implying that cyanide may increase the presence of CAT in leaf cells. However, a marked decline of CAT activity was correlated with an increase in cyanide present in the growth medium.

POD followed a similar trend of CAT. POD activity of willows was severely inhibited by the increase in cyanide concentrations (Table 1). With an increase of cyanide concentrations from 0.30 to 4.72 mg CN L^{-1}, activity of POD of treated plants decreased from 37.18 to 28.26 U g^{-1} FW, whereas POD activity of the non-treated control plants was detected to be 39. 04 U g^{-1} FW.

3.5. Removal of cyanide from hydroponic solution by weeping willows

In the control with cyanide in the absence of plants, negligible changes of cyanide in the solution were found over the entire period of incubation (data not shown), indicating the disappearance of cyanide in solution from the planted systems largely accounted to the uptake by willows. Similar results were also reported before [5,14–16]. The amounts of applied cyanide removed from the hydroponic solution by the presence of weeping willows in all treatments were quantified. Nearly 99% of the aqueous cyanide were removed from the growth media by plants (mean 98.82 %, S.D. 0.309, no. 5). Results showed that large fraction of applied cyanide transported into plant materials in all treatments, and trace amounts of the chemical remained in the hydroponic solutions after 192 h of exposure. This coincided with other relevant findings [8,9,26]. Yu et al. [8] reported that more than 99% of applied cyanide were moved from the solution by weeping willows exposed to up to 3.72 mg CN L^{-1} after 192 h of exposure. In other studies by Larsen et al. [9,26], a large part of cyanide was also observed to be able to remove from the hydroponic solution by basket willows.

3.6. The mass balance of total cyanide

Concentrations of total cyanide in different parts of plant tissues were also determined after 192 h of exposure for different treatments (Fig. 1). The background of total cyanide in non-exposed control trees was 0.023 mg CN kg^{-1} for roots, 0.038 mg CN kg^{-1} for leaves and 0.015 mg CN kg^{-1} for stems ($n = 2$ for all controls), and the concentrations in the respective solutions were below the detection limit of 0.004 mg CN L^{-1}. Cyanide concentrations in roots of the treated plants at the end of cyanide exposure were significantly higher than the controls ($p < 0.05$). A measurable increase in cyanide concentrations in leaves of the treated plants was detected, but no significant difference compared with the non-treated control plants ($p > 0.05$), except for the treatment exposed to 4.74 mg CN L^{-1}. There is

Table 2
Mass balance for cyanide in the hydroponic systems containing weeping willows (*Salix babylonica* L.)

Treatment (mg CN L^{-1})	Mass in solution (μg CN)		Mass in tissues[a] (μg CN)			Metabolism rate (mg CN kg^{-1} d^{-1})
	Initial	Final	Root	Stem	Leaf	
0.30	75.00	1.10 (0.092)	0.22[b] (0.077)	0.69 (0.236)	0.12 (0.038)	0.20 (0.072)
0.59	147.50	1.95 (0.814)	0.31[b] (0.098)	0.36 (0.195)	0.13 (0.088)	0.63 (0.272)
1.19	297.50	4.22 (0.254)	0.62[b] (0.027)	0.40 (0.201)	0.18 (0.013)	0.96 (0.388)
2.37	592.50	4.77 (0.656)	2.12[b] (0.603)	0.51 (0.185)	0.17 (0.048)	1.79 (0.644)
4.74	1185.00	10.60 (3.236)	4.26[b] (0.741)	1.02[b] (0.242)	0.27[b] (0.071)	3.43 (0.698)

The exposure period was 192 h. Values are mean of three replicates, in brackets: standard deviation.
[a] The measured concentrations of total cyanide in plant materials were higher than that of the background cyanide in non-exposed control trees, except for the stems. The background cyanide did not take into account in the mass balance.
[b] Significantly different to the controls on 95% significance level (one-tailed *t*-test).

also no significant difference in cyanide concentrations in stems between the treated plants the controls ($p > 0.05$). Concentrations of the total cyanide in different parts of the plant materials were correlated to the applied cyanide concentratios in the solution significantly, with R^2 values of 0.9634 and 0.7266 for roots and stems, respectively (judged by the critical r for a given n, significant at $\alpha = 0.05$) [25], indicating the uptake and transport of cyanide from the hydroponic solution into plant. However, substantial differences existed in the distribution of cyanide in plant materials, indicating transport and assimilation of cyanide within plant materials.

Due to the leaf portion being exposed to the air, cyanide in water may have been translocated and then evaporated through leaves without metabolism. No cyanide transpired by plant leaves was reported by Ebbs et al. [6]. Therefore, the mass balance for cyanide was calculated using the tissue total cyanide and the solution cyanide data (Table 2). At 0.30 mg CN kg^{-1}, the total cyanide recovered from plant biomass accounted for 1.37% of the cyanide supplied in the growth media, whereas 0.47% was recovered in tissues of plants exposed to 4.74 mg CN kg^{-1}. Most likely, >97% of the applied cyanide was metabolized during the transport within plant materials after 192 h of exposure. This also agreed with earlier findings [6,9,16,26]. It is clearly shown in Fig. 2 that the metabolism of cyanide by plants was in a dose-dependent manner, indicated by the high R^2 0.9921.

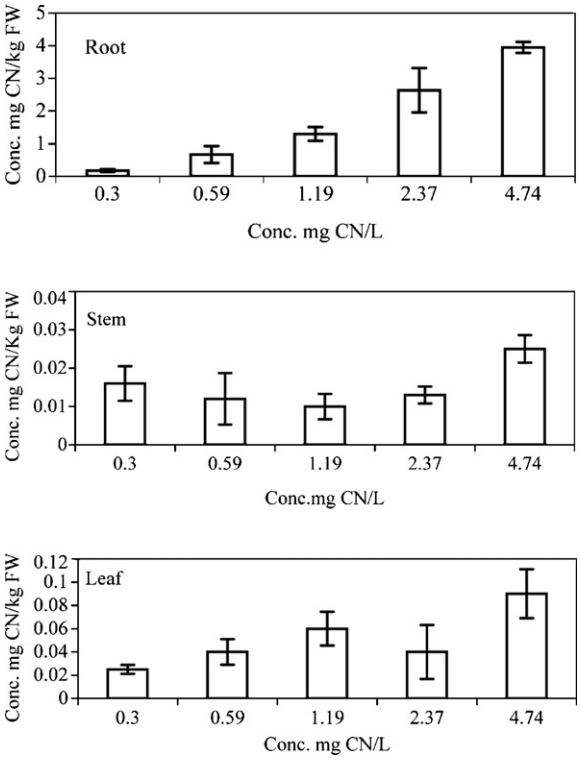

Fig. 1. Measured total cyanide concentrations (mg Cr kg^{-1} FW) in roots, stems and leaves of weeping willows (*Salix babylonica* L.) at different treatment concentrations. The exposure period was 192 h. The values are the mean of three replicates. Vertical lines represent standard deviation; FW, fresh weight.

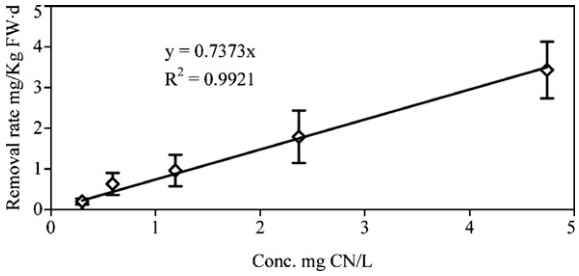

Fig. 2. Cyanide removal rate (mg CN kg^{-1} FW d^{-1}) of weeping willows (*Salix babylonica* L.) at different treatments. The exposure period was 192 h. The values are the mean of three replicates. Vertical lines represent standard deviation.

4. Discussions

The visible lesion in weeping willows due to cyanide exposure was not observed, indicating the doses used in this study did not cause observable deleterious effects on plant physiological functions over the 192-h period of exposure. The results of all measured toxic effects were also analyzed and plotted (data not shown). All linear regressions were significant, except for chlorophyll contents and CAT activity, judged by the critical r for a given n ($\alpha = 0.05$) [25]. Of these selected parameters, the best correlation between the selected parameters and the applied cyanide concentrations was obtained for the soluble protein contents ($R^2 = 0.968$, significant at $\alpha = 0.05$), indicating that soluble protein contents in leaves was the most sensitive to the changes in cyanide doses than the others. The susceptibility of these parameters to the change of cyanide exposure followed the order: soluble proteins >SOD > POD > transpiration rate > chlorophyll b > CAT > chlorophyll a.

In this study, neither visible toxic symptom nor deleterious effects on plant physiological functions was observed due to cyanide application and exposure for the 192 h of exposure. This is largely due to the well-established detoxification systems in willows as well as the enhanced antioxidation. It has been reported that the activity of the enzyme beta-cyanoalanine synthase is several orders of magnitude higher than the cyanide produced by the ethylene synthesis [27]. Indeed, metabolism rates of cyanide by plants increased with increasing external concentrations in this study. Increased activities of antioxidant enzymes were usually correlated with environmental stress. In this study, SOD, CAT and POD activities showed a slight change to cyanide application in most treatments, implying that cyanide taken from the hydroponic solution was quickly metabolized by plants in the presence of the enzyme beta-cyanoalanine synthase, and, in a consequence, the residual cyanide concentrations in plant materials were not high enough to cause stresses to plants. Compared with the controls, higher measured values of transpiration rates, chlorophyll contents and soluble proteins of plants also provide supporting information that cyanide application showed growth-promoting effects on plants. It should be noted that if bioaccumulation of the toxic chemical in plants is beyond the threshold of their detoxifying capacity, the overloaded chemical might lead to toxic effects, and in last consequence to death.

The variation in cyanide application affected both the kinetics of uptake and metabolism and the transpiration rate. Subsequently, the observed change of the of cyanide in hydroponic solution with cyanide supplied could either be due to an increased uptake of the compound into root cells together with the transpiration water, or by diffusion, or due to an increase of metabolism. Both correlations between the metabolic rate and the initial cyanide concentrations (Fig. 2, $R^2 = 0.9921$, significant at $\alpha = 0.01$), and between the initial cyanide concentrations and the transpiration rate were significant (figure not shown, $R^2 = 0.7797$, significant at $\alpha = 0.05$). Additionally, the correlation between the metabolic rate and the transpiration rate was remarkable (figure not shown, $R^2 = 0.7818$, significant at $\alpha = 0.05$). However a partial correlation between cyanide supply, metabolism rate and transpiration rate unveiled that the correlation between initial cyanide concentrations and the metabolic rate, assuming transpiration a constant, would still be 0.9639 (significant at $\alpha = 0.01$). On the other hand, the partial correlation between the metabolic rate and the transpiration rate (assuming cyanide supply a constant) is weaker ($R^2 = 0.0127$) and insignificant (significant at $\alpha = 0.05$). It can be concluded that the uptake of cyanide from the solution is highly dependent on cyanide application rather than transpiration rate. Indeed, it was also found earlier that the removal of leaves, which stops transpiration almost completely, lowered the loss of cyanide from solution insignificantly [9], indicating that cyanide uptake into roots was mainly by diffusion, independently of the uptake of water.

5. Conclusions

Neither deleterious effects on plant physiological functions nor visible toxic symptom was observed due to cyanide application after 192 h of exposure, largely due to the highly active and well functioning detoxifying enzymatic systems in weeping willows. Most selected parameters for toxicity determination showed a dose-dependent manner significantly. Soluble proteins in leaves were noted the most sensitive indictor for the plants exposed to cyanide application. The large fraction of the applied cyanide was removed by plants from the hydroponic solution efficiently and trace amounts of cyanide were detected in plant materials. The metabolic rates of cyanide by plants were linearly increased with the cyanide application. These suggest that cyanide has a beneficial role in plants and phytoremediation is a quite desirable solution of treating environmental sites contaminated with cyanide.

Acknowledgements

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