Available online at www.sciencedirect.com
ScienceDirect
Journal of Hazardous Materials 156 (2008) 300–307
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Effects of available nitrogen on the uptake and assimilation of ferrocyanide and ferricyanide complexes in weeping willows

Xiao-Zhang Yu, Ji-Dong Gu *

Laboratory of Environmental Toxicology, School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, People's Republic of China

Received 27 July 2007; received in revised form 10 December 2007; accepted 10 December 2007
Available online 4 February 2008

Abstract

The effects of different levels of external nitrogen on the uptake, distribution and assimilation of iron cyanide complexes were investigated. Pre-rooted weeping willows (*Salix babylonica* L.) were grown in a hydroponic solution with or without nitrogen and amended with potassium ferrocyanide or potassium ferricyanide at 25.0 ± 0.5 °C for 144 h. Faster uptake of ferrocyanide than ferricyanide was observed in willows grown in the deionized water. Negligible difference in the removal rate between the two chemicals was detected for willows grown in nutrient soultions with or without amendment of nitrogen. The volatilization of ferro- and ferricyanide due to transpiration through plant aerial tissues was below detection level. Less then 20% of the ferrocyanide or ferricyanide taken up from the N-free nutrient solution was recovered in the biomass and majority was accumulated in the roots. In contrast, less than 9.0% of both iron cyanide compelxes taken up was detected in the plant materials of willows grwon in the N-containing nutrient solution and roots were the major sites for accumulation of both chemicals. A large fraction of the ferro- and ferricyanide taken up from the hydroponic solution was assimilated during the transport within plant materials. Willows grown in the N-containing nutrient solution showed a higher assimilation potential for both chemical forms than those grown in the N-free nutrient solution in general. The information collectively suggests that uptake and assimilation mechanisms for ferro- and ferricyanide are largely different in willows; the strength of external nitrogen had a negligible effect on the uptake of both chemicals, while assimilation of ferro- and ferricyanide in plant materials was strongly related to the presence of easily available nitrogen in the hydroponic solution.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Assimilation; Cyanide; Ferrocyanide; Ferricyanide; Nitrogen; Phytoremediation; Uptake; Willows

1. Introduction

Cyanide occurs naturally in plant cells as a by-product during the ethylene synthesis, but anthropogenic activities have drastically altered the distribution and biochemical balance in the environment. It has been estimated that the annual production of hydrogen cyanide (HCN) is 1.4 million metric tons and more than 100,000 tonnes of cyanide enters the environment annually [1], which is a significant contribution to the terrestrial ecosystems.

Cyanide associated with the industrial inputs can frequently exist in two environmentally important oxidation states in soils and groundwater, namely the ferrocyanide $Fe^{II}(CN)_6{}^{4-}$ and the ferricyanide $Fe^{III}(CN)_6{}^{3-}$, which account for more than 97% of the total cyanide [2]. These compounds are environmentally problematic because these complexes are susceptible to photodissociation to release free cyanide when present in the vadose zone or being discharged into surface waters [3,4].

Cyanide is rapidly detoxified by reacting with cysteine to form asparagine by means of the cyanoalanine pathway in vascular plants [5]. In our recent work, metabolic responses and biotransformation of cyanide in weeping willow was studied [6]. The conversion of ferricyanide in yeast cells (*Saccharomyces cerevisiae*) was purportedly either through the ferrireductases involved in iron transfer systems [7] or through the NADH dependent menadione reductase [8]. Although iron cyanide complexes have long been considered membrane impermeable [9], uptake of ferro- and ferricyanide by plants has been investigated [4,10–12] and probably followed by metabolism inside plants [10,11]. Federico and Giartosio [13] confirmed that the existence of a NADH-ferricyanide (O_2) electron transport

* Corresponding author. Tel.: +852 2299 0605; fax: +852 2858 3477/2559 9114.
E-mail address: jdgu@hkucc.hku.hk (J.-D. Gu).

0304-3894/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2007.12.020

system, located within the plasmalemma, can be linked to actively reduction of ferricyanide in maize (*Zea mays* L., var XL 342). It has been proposed that the cyanoalanine pathway has an important role in the nitrogen metabolism [14], while the contribution of this pathway to the metabolism of iron cyanide complexes is unknown. In this study, uptake, assimilation and accumulation of ferro- and ferricyanide in weeping willows were investigated; the influence of N-strength on the uptake and assimilation of both iron cyanide complexes was also examined.

2. Materials and methods

2.1. Trees specimens and exposure regimes

Weeping willows (*S. babylonica* L.) were sampled from the campus of Hunan Agricultural University, PR China. Tree cuttings (40 cm in length) were removed from a mature tree and all cuttings used in this study were obtained from a single tree. They were placed in buckets of tap water at room temperature of 15–18 °C under natural sunlight until new roots and leaves appeared. After a 2-month period of growth, each young rooted cutting was transferred to a 250 mL Erlenmeyer flask filled with approximately 200 mL modified ISO 8692 nutrient solution (Table 1). The flasks were all sealed with cork stoppers and silicon sealant (Dow Chemical Co., Midland, MI) to prevent escape of water, and wrapped with aluminum foil to inhibit potential growth of algae. For each treatment concentration, five replicates were prepared. All flasks were housed in a climate control chamber maintained at a constant temperature of 25.0 ± 0.5 °C under natural sunlight (light:dark cycle 14:10 h). The plants were conditioned for 48 h first to adapt to the new environmental conditions before initiation of the test. Then, the weight of the plant-flask system was measured and recorded individually. The flasks including the tree cuttings were weighed again after 24 h. By doing this way, the transpiration rate of each flask was calculated. Trees with a similar transpiration rate were selected and grouped for the tests. The nutrient solution in each of the flasks was replaced with spiked solution. Potassium ferricyanide [$K_3Fe(CN)_6$] or potassium ferrocyanide [$K_4Fe(CN)_6$] of analytical grade with ≥95% purity were used. It should be noted that 1 mg $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ equals to 0.474 and 0.424 mg CN, respectively. The concentration of ferro- and ferricyanide in spiked solution was therefore 8.37 (±1.14) and 9.32 (±0.44) mg CN L^{-1} for ferro- and ferricyanide, respectively.

Seven different treatments were prepared for each testing chemical: (1) deionized water (control); (2) 20% strength N-free nutrient solution (S-1); (3) 60% strength N-free nutrient solution (S-2); (4) 100% strength N-free nutrient solution (S-3); (5) 20% strength N-containing nutrient solution (S-4); (6) 60% strength N-containing nutrient solution (S-5); (7) 100% strength N-containing nutrient solution (S-6). Additionally, two sets of controls were also conducted: one was with ferro- or ferricyanide, but without plants to quantify the loss of testing chemicals during handing, hydrolysis and/or degradation by microorganisms; the other was with ferro- or ferricyanide and willows to measure whether the applied iron cyanides dissociated in solutions in the presence of plants before uptake.

Table 1
Composition of the modified ISO 8692 nutrient solution used in this study

Macronutrients (μmol L^{-1})		Micronutrients (nmol L^{-1})	
$NaNO_3$[a]	2823.9	H_3BO_3	2992.1
$MgCl_2·6H_2O$	59.0	$MnCl_2·4H_2O$	2097.0
$CaCl_2·2H_2O$	122.4	$ZnCl_2$	22.0
$MgSO_4·7H_2O$	60.9	$CoCl_2·6H_2O$	6.3
KH_2PO_4	246.0	$CuCl_2·2H_2O$	0.1
$NaHCO_3$	1785.5	$NaMoO_4·2H_2O$	28.9

[a] Used in solutions 4–6.

2.2. Determination of the transpiration rate

Inhibition of transpiration is a rapid measure for the toxic effect of a chemical or a substrate to trees to be analyzed [15]. The effect of iron cyanide complexes was quantified by measuring the transpiration rate of willows in flasks subject to a range of treatment conditions in the hydroponic solutions. The weight loss of the plant-flask system was obtained and expressed as the transpiration rate.

2.3. Determination of the assimilation rates of iron cyanide complexes

The assimilation capacity of iron cyanide complexes v_p (μg CN g^{-1} FW day^{-1}) was calculated from

$$v_p = \frac{M_{(intial)} - M_{(final)} - M_{(root)} - M_{(stem)} - M_{(leaf)}}{W_{(plant)}\Delta T}$$

where $M_{(initial)}$, $M_{(final)}$, $M_{(root)}$, $M_{(stem)}$ and $M_{(leaf)}$ are the total cyanide (μg) in hydroponic solution and in different plant materials. $W_{(plant)}$ is the biomass of the plant (g), and ΔT is the time period of exposure (day).

2.4. Determination of translocation efficiency

The translocation efficiency (τ) as the fraction that, after root uptake, is successfully translocated to upper parts of plants as defined by Meers et al. (2004):

$$\tau(\%) = \frac{M_{(leaf)}FW_{(leaf)} + M_{(stem)}FW_{(stem)}}{M_{(root)}FW_{(root)} + M_{(leaf)}FW_{(leaf)} + M_{(stem)}FW} \times 100$$

where $M_{(root)}$, $M_{(stem)}$ and $M_{(leaf)}$ are the total cyanide concentration in different plant materials, and $FW_{(root)}$, $FW_{(stem)}$ and $FW_{(leaf)}$ are the fresh weight production in plant materials.

2.5. Chemical analysis

Total cyanide in water: Total cyanide is the sum of easily liberated cyanide (HCN and CN^-) and complexed cyanide. The total cyanide in the solution was analyzed by a standard method (State Environmental Protection Administration of PR China). Ten milliters of 1% NaOH were added into the reservoir vessel of the distillation unit. Five milliters of the spiked solution were placed in a 500 mL round bottom flask, and then 200 mL

of distilled water were added. Then 10 mL of sodium ethylenediamine tetraacetate (EDTA) 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. All samples were analyzed within a maximum holding time of 4 h.

One to five milliters of solution samples were pippetted into a 25 mL colorimetric cylinder (depending on the concentraions of cyanide in the samples), and 0.1% NaOH was added to the mark of 10 mL. Then 5.0 mL of a buffer solution consisting of potassium dihydrogen phosphate (34.0 g L^{-1}) and sodium phosphate (35.5 g L^{-1}) 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. Five milliters of the colour reagent consisting of isonicotinic acid and 3-methyl-1-phenyl-5-pyrazolone (a mixture of isonicotinic acid and 3-methyl-1-phenyl-5-pyrazolone, 5:1, v/v) were then added. Five grams of isonicotinic acid were dissolved in 240 mL of 2% NaOH solution and deionized water was added to the mark of 1000 mL. 3-Methyl-1-phenyl-5-pyrazolone was made by dissolving 2.5 g of the chemical in 200 mL *N*,*N*-dimethyl formamide. The two solutions were mixed just before use each time. 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 absorbance of light at 638 nm was measured in a cell of optical path length of 10 mm against deionized 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 against a standard solution of $AgNO_3$ (0.0100 mol L^{-1}), which was also calibrated with a standard solution of NaCl (0.0100 mol L^{-1}) (standard method of SEPA, PR China). The detection limit of this method was determined to be 0.004 mg CN L^{-1}, depending on the volume of the sample used. The sample preparation methods used in this study were also checked against spiking samples of certified solution standards and the mean recovery was 98.46% ($n = 10$).

Total cyanide in plant materials: The total cyanide in plant materials was analyzed based on the method by Yu et al. [12]. Plant materials from the treated and the non-treated plants were harvested after 144 h of experiments. Fresh plant biomass (2.5–15 g FW, depending on the harvested weight of plant materials) cut into small pieces were used instead of 5 mL of the spiked sample. The remaining procedures were identical to those described earlier.

2.6. Phytovolatilization of iron cyanide complexes

Volatilization of iron cyanide complexes due to plant transpiration was measured using a modified test chamber system. The treated plants were prepared as described above with the entire 250 mL Erlenmeyer flask-plant and were enclosed in a glass chamber (20 cm × 20 cm × 50 cm) with air flowing through at 25 °C. The tube at the outflow of the vessel was connected to a gas trap containing 5 mL of 1% sodium hydroxide to trap airborne cyanide. The gas trap tube was wrapped with aluminum foil and changed daily, after which all gas tubes were analyzed for total cyanide. The duration of this test was 144 h.

2.7. Statistical methods

ANOVA test and Tukey's multiple comparison tests were used to determine the statistical significance at the 0.05 level between plant performances.

3. Results

3.1. Uptake of ferrocyanide from hydroponic solution by willows

Fig. 1 shows the measured concentrations of total cyanide in hydroponic solution at different treatments after 144 h of exposure. In the control with ferrocyanide in the absence of plants, negligible changes of total cyanide in solutions were found over the entire exposure period, indicating that the disappearance of ferrocyanide from the hydroponic solution within the planted systems could be accounted for by the uptake by willows. The total cyanide concentration in solution was reduced from 9.66 to 7.95 (±1.25) mg CN L^{-1} by weeping willows grown in the deionized water amended with ferrocyanide (controls), which accounted for 31.82% reduction of the applied ferrocyanide in the hydroponic solution. Compared to the controls, a slightly lower removal rate was found in other treatments. Approximately 26% of the initial ferrocyanide was removed from the N-free hydroponic solution by plants, whereas between 19.78% and 21.24% of the supplied ferrocyanide was removed from the N-containing nutrient solution after the 144 h of exposure. The difference in the removal of ferrocyanide in the hydroponic solution between the treatments with nitrogen supply and those without nitrogen supply was not significant ($p > 0.05$). This observation indicated that the presence of external nitrogen in the plant growth nutrient media did not show a significant impact on the removal of ferrocyanide from the hydroponic solution.

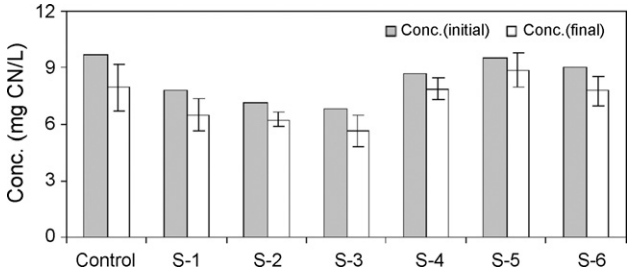

Fig. 1. Measured total cyanide concentration (mg CN L^{-1}) in the hydroponic solution spiked with ferrocyanide at the beginning and end of the experiment. The exposure period was 144 h. The values are the mean of five replicates for samples. Vertical bars represent standard deviation.

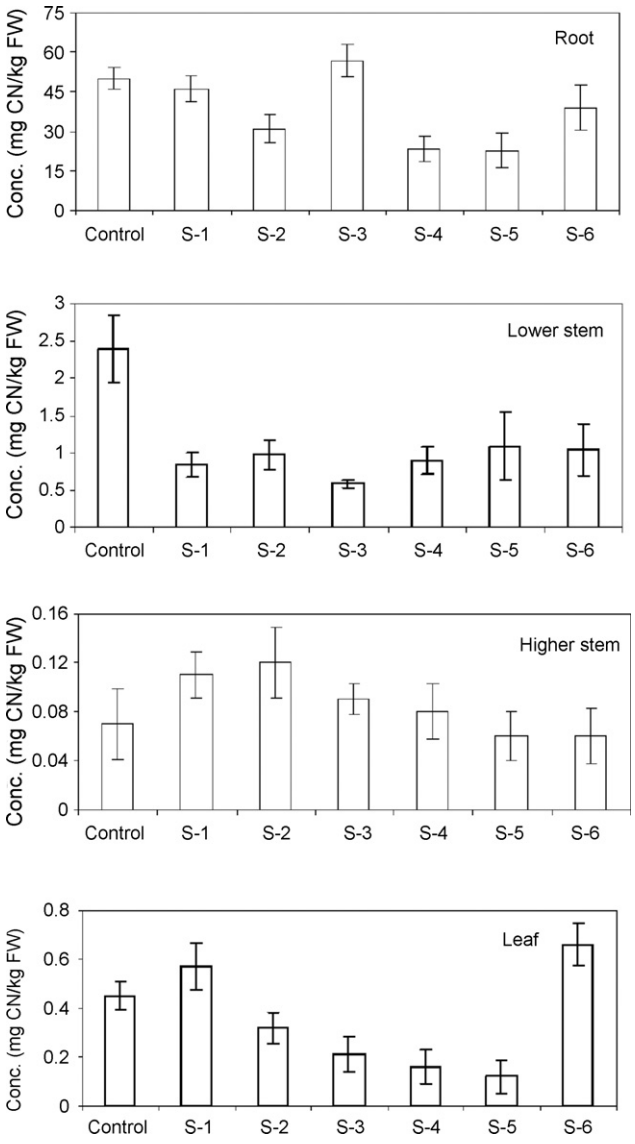

Fig. 2. Measured total cyanide concentration (mg CN kg^{-1} FW) in plant materials of weeping willows (*Salix babylonica* L.) exposed to ferrocyanide. The exposure period was 144 h. The values are the mean of five replicates for samples. Vertical bars represent standard deviation. FW: fresh weight.

3.2. Distribution of ferrocyanide in plant materials

Fig. 2 presents the concentrations of total cyanide in roots, lower stems, higher stems and leaves of weeping willows grown in solutions with different treatments. The background cyanide in non-exposed control trees was 0.023 mg CN kg^{-1} for roots, 0.015 mg CN kg^{-1} for stems and 0.038 mg CN kg^{-1} for leaves ($n = 2$ for all controls), and the concentrations in the respective control solutions were below the detection limit of 0.004 mg CN L^{-1}. Cyanide was detected in all parts of plant materials in all treatment groups and all measured concentrations were elevated in comparison with the background cyanide, confirming the uptake of ferrocyanide into plant biomass from the hydroponic solution and also the translocation within plant materials. However, substantial differences existed in the distribution of ferrocyanide in different parts of plant tissues.

For the controls (weeping willows grown in the deionized water with ferrocyanide), the highest concentration was found in the roots (49.88 ± 4.29 mg CN kg^{-1}), followed by the lower stems with a value of 2.39 (± 0.44) mg CN kg^{-1}. The lowest cyanide concentration in the biomass was in the higher stems (0.07 ± 0.02 mg CN kg^{-1}). When grown in the N-free nutrient solution amended with ferrocyanide, a significant difference in cyanide concentrations between the treatments due to the strength of N-free nutrient solutions was found in roots and also in higher stems ($p < 0.05$), while a measurable difference in cyanide concentration was detected in lower stems and in leaves ($p > 0.05$). On the other hand, a remarkable difference in the cyanide concentrations in biomass of weeping willows exposed to the N-containing nutrient solution amended with ferrocyanide was found ($p < 0.05$), with one exception in which the cyanide concentration in leaves between all the treatments was identical ($p > 0.05$).

After uptake from the hydroponic solution and translocation to leaves, ferrocyanide may have been volatilized. However, no cyanide above the detection limit was detected in the trap in the presence of willows in this study. The same result was reported in an early study by Yu et al. [12]. Table 2 illustrates the distribution of ferrocyanide in solution and of that accumulated in plant materials after 144 h of exposure. Between 11.67% and 20.82% of the ferrocyanide taken up from the N-free hydroponic solution was recovered in plant materials of weeping willows, whereas 16.73% was detected in plant materials of the control trees. Root was the major site for ferrocyanide accumulation in the control trees, which accounted for 68.26% of the total ferrocyanide accumulated in biomass. The ferrocyanide associated with the lower stems was 29.55% and that accumulated in the higher stems and leaves of the control trees was only trace quantities. Compared to the control trees, more ferrocyanide was accumulated in the roots: between 70.03% and 85.04% of the ferrocyanide was accumulated in the roots; less ferrocyanide was found in the lower stems. It is of interest to note that a significant decrease in the ferrocyanide accumulated in biomass was found in willows due to the application of external nitrogen in plant growth media ($p < 0.05$). Between 56.25% and 65.77% of the ferrocyanide accumulated were detected in roots, followed by the lower stems (31.19–39.50%) and small amounts were found in the higher stems and leaves of willows grown in the N-containing solution.

All loss of ferrocyanide within the planted system was largely due to assimilation by plants. This is consistent with several earlier findings [10–12]. The calculated assimilation rates of ferrocyanide are shown in Table 2. The assimilation rate of ferrocyanide was 2.82 (± 0.18) mg CN kg^{-1} day^{-1} for the control willows. A clear decrease in the assimilatory rate of ferrocyanide was observed in willows grown in the N-free nutrient solution as a function of increasing the strength of nutrient solutions, while no significant difference in the assimilatory rate was found in plants due to external nitrogen application ($p > 0.05$). Results indicated that other nutrient elements in plant growth media resulted in a significant decrease in the assimilatory rate of ferrocyanide by weeping willows, and the presence of easily available nitrogen had a minor impact on ferrocyanide assimilation in plants.

Table 2
Distribution of the applied ferrocyanide in plant materials[a]

Treatment	Total cyanide in solution (μg)		Total cyanide in plant tissues (μg)				Total cyanide recovery (%)	Translocation efficiency (%)	Assimilation rate (mg CN kg^{-1} day^{-1})
	Initial	Final	Root	Lower stem	Higher stem	Leaf			
Control	2415.00	1647.09 (108.87)	79.99 d (18.11)	34.63 b (10.75)	0.85 a,b (0.21)	1.71 b (0.18)	16.73 c,d (2.54)	32.97 a,b,c (10.01)	2.82 a (0.18)
S-1	1940.00	1427.66 (100.94)	48.47 b,c,d (6.52)	10.6 a (2.08)	0.98 a,b (0.19)	1.27 a,b (0.09)	11.67 a,b,c (2.34)	21.02 a,b (2.74)	2.16 a (0.49)
S-2	1782.50	1318.42 (105.85)	43.56 a,b,c (8.29)	15.84 a (2.91)	1.65 b (0.46)	1.14 a,b (0.28)	13.83 b,c,d (2.86)	30.09 a,b,c (3.17)	1.52 a (0.14)
S-3	1702.50	1259.47 (198.76)	72.08 c,d (10.47)	10.65 a (1.78)	1.24 a,b (0.65)	0.79 a,b (0.20)	20.82 d (6.6)	15.95 a (3.86)	1.29 a (0.42)
S-4	2177.50	1715.63 (96.27)	12.36 a (3.90)	8.71 a (2.89)	0.63 a (0.29)	0.35 a (0.11)	5.73 a (1.06)	44.58 c (11.95)	2.81 a (0.84)
S-5	2375.00	1906.15 (192.70)	15.84 b (5.52)	10.11 a (2.36)	0.51 a (0.13)	0.25 a (0.04)	6.66 a,b (2.17)	41.59 c (9.18)	2.67 a (0.34)
S-6	2257.50	1791.20 (103.81)	20.12 c (4.36)	9.54 a (2.41)	0.44 a (0.10)	0.29 a (0.13)	8.46 a,b (2.58)	35.32 b,c (10.27)	2.79 a (0.54)

Exposure period: 7 days; the values are the mean of five replicates; in brackets: standard deviation.
[a] One single factor ANOVA. The same letters (a, b, c and d) are not significantly different at the 5% level according to Tukey's multiple range test.

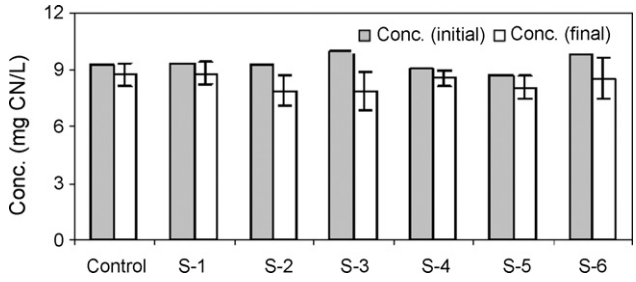

Fig. 3. Measured total cyanide concentration (mg CN L^{-1}) in the hydroponic solution spiked with ferricyanide at the beginning and end of the experiment. The exposure period was 144 h. The values are the mean of five replicates for samples. Vertical bars represent standard deviation.

3.3. Uptake of ferricyanide from hydroponic solution by willows

Fig. 3 gives the changes of total cyanide concentrations in hydroponic solution spiked with ferricyanide. In the control with ferricyanide in the absence of plants, the change of total cyanide in solutions was also negligible over the entire exposure period. The total cyanide concentration declined from 9.19 to 8.74 (±0.59) mg CN L^{-1} by weeping willows grown in the deionized water spiked with ferricyanide, which accounted for 21.12 (±5.02)% removal of the applied ferricyanide. A significant increase in the removal of ferricyanide from hydroponic solution was found for weeping willows grown in the N-free nutrient solution with an increase of the strength of nutrient solution ($p < 0.05$). Between 20.10 (±5.57)% and 34.00 (±7.42)% of the applied ferricyanide was removed from the hydroponic solution. A measurable difference in the removal of ferricyanide between the N-containing treatments was found ($p < 0.05$) in which between 17.49 (±3.08)% and 22.29 (±5.42)% of the applied ferricyanide were removed from the hydroponic solution by willows. This observation suggested that the presence of other nutrient elements rather than nitrogen in the plant growth nutrient media had a positive impact on the removal of ferricyanide from the hydroponic solution by plants.

3.4. Distribution of ferricyanide in plant materials

The total cyanide concentrations in plant materials of weeping willows exposed to ferricyanide are presented in Fig. 4. Elevated cyanide concentrations were found in all parts of plant tissues from all treatment groups. When willows were grown in the deionized water amended with ferricyanide, the highest concentration was found in the roots (65.27 ± 12.17 mg CN kg^{-1}), followed by the lower stems (1.65 ± 0.36 mg CN kg^{-1}), and the lowest were in the higher stems (0.02 ± 0.007 mg CN kg^{-1}). A significant difference in the cyanide concentration was only found in roots of willows grown in the N-free nutrient solution ($p < 0.05$), while a remarkable difference in the cyanide concentration was found in all parts of plant materials due to the application of external nitrogen in nutrient solution amended with ferricyanide ($p < 0.05$).

No cyanide above the detection limit was trapped in the gas trap for the treatment with ferricyanide in the testing chamber

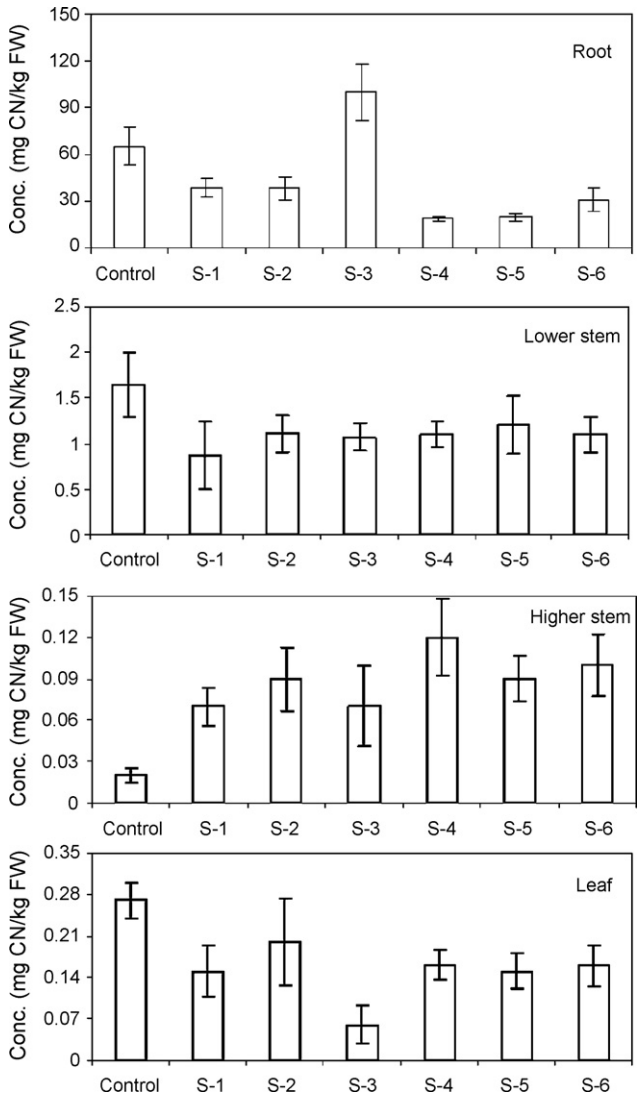

Fig. 4. Measured total cyanide concentration (mg CN kg^{-1} FW) in plant materials of weeping willows (*Salix babylonica* L.) exposed to ferricyanide. The exposure period was 144 h. The values are the mean of five replicates for samples. Vertical bars represent standard deviation. FW: fresh weight.

system and the distribution of ferricyanide accumulated in plant materials after 144 h of exposure is summarized in Table 3. The ferricyanide taken up from the deionized water (controls) was recovered to be 22.13% in the biomass, whereas a similar level of recovery rate was found in willows grown in the N-free nutrient solution. One exception is that a significantly lower recovery was found in willows exposed to the 60% strength N-free nutrient solution. Majority (78.42%) of the ferricyanide accumulated in biomass was associated with roots. Ferricyanide accumulated in the lower stems accounting for 20.48% of the total and trace amounts were detected in the higher stems and leaves, respectively. More ferricyanide was found in the roots of plants grown in the 100% strength N-free nutrient solution in which approximate 90% of the ferricyanide accumulated in biomass was recovered. A significant decrease in the ferricyanide accumulated in biomass was found in willows grown in the N-containing nutrient solution ($p < 0.05$), where between 6.39% and 7.24%

Table 3
Distribution of the applied ferricyanide in plant materials[a]

Treatment	Total cyanide in solution (μg)		Total cyanide in plant tissues (μg)				Total cyanide recovery (%)	Translocation efficiency (%)	Assimilation rate (mg CN kg^{-1} day^{-1})
	Initial	Final	Root	Lower stem	Higher stem	Leaf			
Control	2297.50	1811.50 (115.38)	80.79 b (12.48)	21.10 b (4.75)	0.25 a (0.04)	1.01 b (0.26)	22.13 c (5.56)	23.29 a,b (14.11)	1.75 a (0.51)
S-1	2337.50	1867.06 (130.16)	56.71 a,b (6.20)	12.17 a,b (3.26)	0.79 a,b (0.21)	0.48 a (0.19)	15.67 b,c (3.59)	18.46 a,b (6.87)	1.80 a (0.50)
S-2	2297.50	1663.67 (92.74)	44.83 a,b (10.09)	18.06 a,b (4.84)	1.29 b (0.41)	0.51 a (0.18)	12.50 a,b (2.85)	32.22 b,c (8.83)	2.20 a (0.74)
S-3	2495.00	1646.78 (125.24)	145.90 c (27.87)	15.99 a,b (3.91)	0.82 a,b (0.24)	0.32 a (0.04)	21.12 c (4.05)	11.33 a (4.33)	2.77 a (0.78)
S-4	2257.50	1862.58 (69.49)	15.37 a (27.87)	9.63 a (0.98)	0.79 a,b (0.25)	0.35 a (0.12)	6.84 a (1.00)	42.56 c (8.54)	2.44 a (0.56)
S-5	2177.50	1751.29 (64.08)	16.79 a (6.47)	12.23 a,b (2.66)	0.86 a,b (0.32)	0.25 a (0.04)	7.24 a (2.23)	45.72 c (10.51)	2.49 a (0.35)
S-6	2455.00	1908.03 (130.30)	25.66 a (7.14)	9.69 a (1.48)	0.70 a,b (0.22)	0.29 a (0.13)	6.29 a (1.48)	30.40 b,c (8.51)	4.15 b (1.02)

Exposure period: 7 days; the values are the mean of five replicates; in brackets: standard deviation.
[a] One single factor ANOVA. The same letters (a, b, c and d) are not significantly different at the 5% level according to Tukey's multiple range tests.

of the ferricyanide taken up were recovered in the biomass of willows grown in the N-containing hydroponic solution: roots being the major sites for ferricyanide accumulation as well.

The calculated assimilation rates for ferricyanide are shown in Table 3. The assimilatory rate of ferricyanide was 1.75 (±0.51) mg CN kg^{-1} day^{-1} for the control trees, whereas a measurable increase in the rate was observed for willows grown in the N-free nutrient solution with an increase of the strength of N-free nutrient solutions. A significantly higher assimilatory rate was found for plants grown in the 100% N-containing solution ($p < 0.05$), in which the rate was 2.57-fold higher than that of plants grown in the deionized water. Results indicated that the assimilation of ferricyanide by weeping willows is not only related to external nitrogen supply, but also to other nutrient elements present in the plant growth media.

3.5. Response of plant transpiration to the exposure of iron cyanide complexes

Fig. 5 shows the absolute transpiration of weeping willows. A significant difference in the transpiration rate was found for the treatments exposed to ferrocyanide ($p < 0.05$). For the trees exposed to ferricyanide, the transpiration rate between the seven treatments was also significantly different ($p < 0.05$). However, higher transpiration rates were found for the willows grown in the N-free nutrient solutions amended with ferro- or ferricyanide than those grown in N-containing nutrient solutions with testing chemicals only. There was also no significant difference in the plant growth (biomass) between all the treatments exposed to both iron cyanide complexes (data not shown) after the entire period of incubation. Symptoms of chlorosis of leaves were not observed in any plants.

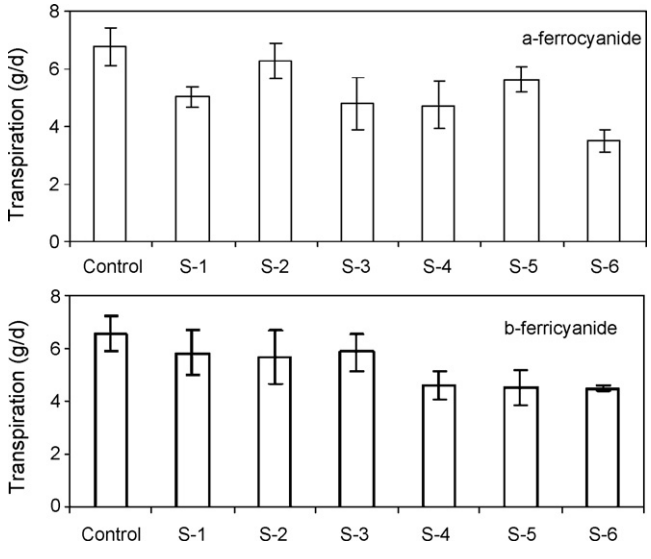

Fig. 5. Measured plant transpiration rate (g day^{-1}) of weeping willows (*Salix babylonica* L.) exposed to ferrocyanide or ferricyanide under different strength of nutrient solution. The values are the mean of five replicates. Vertical bar represents standard deviation.

4. Discussion

In reality there is always a possibility that nitrogen is presented at the contaminated sites, which may affect the phytoremediation efficiency for the targeted chemicals. In this study, approximately 20% of the applied ferro- and ferricyanide in the hydroponic solution was removed by weeping willows over a 144 h of exposure, which is consistent to other earlier findings [4,10–12]. This observation indicated that easily available nitrogen had a negligible impact on the uptake of iron cyanide complexes by willows. It is noted that when plants were grown in the deionized water spiked with ferro- or ferricyanide, more ferrocyanide from the hydroponic solution was removed by willows than ferricyanide, implying that willows can take up both iron cyanide complexes by dissimilar mechanisms and the conversion of ferricyanide to ferrocyanide in hydroponic solution prior to uptake by roots was unlikely to take place.

Less than 9% of the ferro- and ferricyanide taken up from the N-containing hydroponic solution was recovered in the biomass of willows, whereas a higher recovery (approximate 15%) of both iron cyanide complexes was detected in the plant materials of willows grown in the N-free nutrient solution. This indicated that willows grown in N-containing nutrient solutions showed a higher potential for assimilating both iron complexes than those grown in the N-free nutrient solution. Among the ferricyanide treatments supplied with additional nitrogen, significantly higher assimilation rate for ferricyanide was found for willows grown in the 100% N-containing nutrient solution compared with other two treatments ($p < 0.05$).

Willows assimilate iron through solubilization of Fe^{3+} by extracellular acidification, reduction of Fe^{3+} to Fe^{2+} by a plasmamembrane redox system named ferrireductase first, uptake of Fe^{2+} can then be achieved by a specific transporter located in membrane [17–19]. This mechanism requires the obligatory reduction of extracellular Fe^{3+}-chelate complexes before the splitting of the complex and then uptake of the released Fe^{2+}. The conversion of ferricyanide to ferrocyanide or free cyanide is likely to occur prior to the uptake by plant roots. However, Larsen and Trapp [11] observed that there was little support from the experimental results that ferricyanide was taken up as Fe^{2+} ion after reduction in solution. Additionally, in other previous findings willows showed a significantly higher removal capacity for free cyanide [6,10,16]. Indeed, the dissociation of ferri- or ferrocyanide to free cyanide was undetectable within the planted systems in out study.

Accumulation of free cyanide in healthy trees was not observed [16]. In this study, significantly elevated concentrations of cyanide were detected in all parts of plant materials from all treatment groups comparing with the background level, implying that both iron cyanide complexes detected in plant tissues were probably still in the same original chemical complex form. Larsen and Trapp [11] also reported the same information. Additionally, the ^{15}N was not detected in willow tissues as cyanide when ^{15}N labeled ferrocyanide was used [10]. Therefore, we have a good reason to postulate that the metabolic pathways of free cyanide and the iron cyanide complexes are distinctively

different and willows can directly assimilate both ferro- and ferricyanide.

Here, it is also interesting to discuss the relationship between transport and assimilation of both iron cyanide complexes in plant materials. The highest root concentration factor (the concentration ratio between roots and initial solution, RCF) was found at the treatment with 100% strength of N-free nutrient solution amended with ferrocyanide, at which the lowest assimilation rate and translocation efficiency of ferrocyanide were detected, indicating that the external nitrogen resulted not only in poor transport of ferrocyanide within plant materials, but also in a decrease of ferrocyanide assimilation in willows. Therefore, it is suggestive that there is a very close relation between transport and assimilation of ferrocyanide in plant materials in the absence of external nitrogen in plant growth media. When plants were grown in N-containing solutions amended with ferrocyanide, a slight difference in RCF, translocation efficiency, and assimilation rate between the treatments was found, implying that the translocation efficiency and assimilation rate of ferrocyanide in plant materials were independent upon the doses of external nitrogen present in the plant growth media. On the other hand, the highest RCF and assimilation rate were all found at the treatment with 100% strength of N-free nutrient solution amended with ferricyanide, at which the lowest translocation efficiency was achieved, indicating that other nutrient elements in the N-free nutrient solutions had a positive effect on the assimilation of ferricyanide, but inhibited the transport of ferricyanide within plant materials. When willows were grown in N-containing nutrient solutions, an identical scenario was also found. This suggests that the external nitrogen in the plant growth media resulted in a positive role in the assimilation of ferricyanide in willows, but a negative impact on the transport of ferricyanide within plant materials.

5. Conclusions

Weeping willows were able to remove both iron cyanide complexes from the hydroponic solution. N-strength in the nutrient solution did not show a significant impact on the uptake of both ferro- and ferricyanide. Less cyanide was recovered in biomass of willows grown in the N-containing nutrient solution than those grown in the N-free nutrient solution. Roots were the dominant sites for accumulation of both chemicals. The volatilization of ferro- and ferricyanide due to transpiration was undetectable. Majority of the ferro- and ferricyanide taken up from the hydroponic solution could be assimilated by plants. Willows grown in the N-containing nutrient solution showed a higher assimilation potential for both chemicals than those grown in the N-free nutrient solution generally. The information collectively suggests that uptake and assimilation mechanisms for ferro- and ferricyanide are largely different in willows.

Acknowledgements

This work was supported by a Ph.D. studentship from The University of Hong Kong. Thanks to Tian-Peng Li and Li-Qun Xing for their technical assistance.

References

[1] T. Mudder, M. Botz, A guide to cyanide, Min. Environ. Manage. 9 (2001) 8–12.
[2] T.L. Theis, T.C. Young, M. Huang, K.C. Knutsen, Leachate characteristics and composition of cyanide-bearing wastes from manufactured gas plants, Environ. Sci. Technol. 28 (1994) 99–106.
[3] P. Kjeldsen, Behaviour of cyanides in soil and groundwater: a review, Wat. Air Soil Pollut. 115 (1999) 279–307.
[4] M. Samiotakis, S.D. Ebbs, Possible evidence for transport of an iron cyanide complex by plants, Environ. Pollut. 127 (2004) 169–173.
[5] K. Manning, Detoxification of cyanide by plants and hormone action, in: Ciba Foundation Ed., Cyanide Compounds in Biology, John Wiley & Sons, Chichester, UK, 1988, 92–110.
[6] X.Z. Yu, J.-D. Gu, S. Liu, Biotransformation and metabolic responses of cyanide in weeping willows, J. Hazard. Mater. 147 (2007) 838–844.
[7] E. Lessuisse, M. Casteras-Simon, P. Labbe, Ferrireductase activity in *Saccharomyces cerevisiae* and other fungi: colorimetric assays on agar plates, Anal. Biochem. 226 (1995) 377–379.
[8] Y. Yashiki, S. Yamashoji, Extracellular reduction of menadione and ferricyanide in yeast cell suspension, J. Ferment. Bioeng. 82 (1996) 319–321.
[9] J.L. Roustan, J.M. Sablayrolles, Feasibility of measuring ferricyanide reduction by yeast to estimate their activity during alcoholic fermentation in wine-making conditions, J. Biosci. Biotechnol. 96 (2003) 434–437.
[10] S.D. Ebbs, J. Bushey, S. Poston, D. Kosma, M. Samiotakis, D. Dzombak, Transport and metabolism of free cyanide and iron cyanide complexes by willow, Plant Cell Environ. 26 (2003) 1467–1478.
[11] M. Larsen, S. Trapp, Uptake of iron cyanide complexes into willow trees, Environ. Sci. Technol. 40 (2006) 1956–1961.
[12] X.Z. Yu, P.H. Zhou, Y.M. Yang, The potential for phytoremediation of iron cyanide complex by willows, Ecotoxicology 15 (2006) 461–467.
[13] R. Federico, C.E. Giartosio, A transplasmamembrane electron transport system in maize, Plant Physiol. 73 (1983) 182–184.
[14] A. Maruyama, K. Saito, K. Ishizawam, Beta-cyanoalanine synthase and cysteine synthase from potato: molecular cloning, biochemical characterization, and spatial and hormonal regulation, Plant Mol. Biol. 46 (2001) 749–760.
[15] S. Trapp, K.C. Zambrano, K.O. Kusk, U. Karlson, A phytotoxicity test using transpiration of willows, Arch. Environ. Contam. Toxicol. 39 (2000) 154–160.
[16] M. Larsen, A. Ucisik, S. Trapp, Uptake, metabolism, accumulation and toxicity of cyanide in willow trees, Environ. Sci. Technol. 39 (2005) 2135–2142.
[17] H. Marschner, V. Römheld, Strategies of plants for acquisition of iron, Plant Soil 165 (1994) 261–274.
[18] M.L. Guerinot, Y. Ying, Iron: nutritious, noxious, and not readily available, Plant Physiol. 104 (1994) 815–820.
[19] D. Eide, Molecular biology of iron and zinc uptake in eukaryotes, Curr. Opin. Cell Biol. 9 (1997) 573–577.