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

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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.

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

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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