Plant uptake of cyanide

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INSTITUTE OF ENVIRONMENT & RESOURCES

Plant uptake of cyanide

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Ph.D. Thesis

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Institute of Environment & Resources
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Plant uptake of cyanide

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Preface

The work in this Ph.D. thesis has been funded by a DTU Ph.D. grant. The study has been conducted at Institute of Environment & Resources, Technical University of Denmark, from February 2002 until March 2005 with Dr. Stefan Trapp as main supervisor and associate professor Dieke Postma as co-supervisor. The thesis is based on three journal papers:

(i) **Larsen, M.**, Trapp, S., Pirandello, A., 2004. Removal of cyanide by woody plants. Chemosphere, 54, 325-333.

(ii) **Larsen, M**., Ucisik, A. S., Trapp, S., 2005. Uptake, metabolism, accumulation and toxicity of cyanide in willow trees. Environ. Sci. Technol. 39, 2135-2142.

(iii) **Larsen, M.**, Trapp, S., 2006. Uptake of iron cyanide complexes into willow trees. Environ. Sci. Technol., in press.

The papers are not included in this www-version but can be obtained from the Library at the Institute of Environment & Resources, Bygningstorvet, Building 115, Technical University of Denmark, DK-2800 Kgs. Lyngby (library@er.dtu.dk).

In addition to these, the following conference abstracts and articles have been published partly or fully based on this thesis:

Larsen, M., Trapp, S., 2004. Determination of *Michaelis-Menten* kinetics for the removal of cyanide by plants. Abstract. In: Environmental science solutions: A Pan-European perspective. SETAC Europe 14th annual meeting, Prague, Czech Republic 18-22 April 2004. Abstracts, p. 248. SETAC, Brussels.

Trapp, S., Ücisik, A. S., Romano, P. D. & **Larsen, M.,** 2004. The role of plants and bacteria in phytoremediation. Abstract. In: International NATO advanced research workshop "Bioremediation of soils contaminated with aromatic compounds: Effects of rhizosphere, bioavailability, gene regulation and stress adaptation, Tartu, Estonia, 1-3 July 2004.

Larsen, M., Trapp, S., 2003. Risk assessment of cyanide contaminated gas work soil. Abstract. In: Understanding the complexity of environmental issues. A way to sustainability. SETAC Europe 13th annual meeting, Hamburg, Germany 27 April - 1 May 2003. Abstracts, p. 203. SETAC, Brussels.

Larsen, M., Trapp, S., 2003. Determination of Michaelis-Menten kinetics for the removal of cyanide by plants. Abstract. In: International symposium biochemical interactions of microorganisms and plants with technogenic environmental pollutants, Saratov, Russia, 28-30 July 2003, Abstract Book, p. 23. Saratov University Publishing House, Saratov.

Sismilich, M., Suwannaeatana, S., **Larsen, M.,** Trapp, S., 2003. Results from a rapid test of acute toxicity to trees. Poster P44. In: COST 837. Achievements and prospects of phytoremediation in Europe. Final workshop and management committee meeting, 15-18 October 2003, Vienna. Book of Abstracts, IWGA-SIG, Dept. for Sanitary Engineering and Water Pollution Control, Vienna.

Trapp, S., **Larsen, M.,** 2003. Phytoremediation of cyanide-polluted soils and waste waters. Poster P55. In: COST 837. Achievements and prospects of phytoremediation in Europe. Final workshop and management committee meeting, 15-18 October 2003, Vienna. Book of Abstracts, IWGA-SIG, Dept. for Sanitary Engineering and Water Pollution Control, Vienna.

Trapp, S., **Larsen, M.**, Pirandello, A., Danquah-Boakye,J., 2003. Feasibility of cyanide elemination using plants. Technical note. European Journal of Mineral Processing and Environmental Protection, 3, (128), 137.

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Copenhagen, March 2005

Morten Larsen

Abstract

Cyanide (CN) was a by-product of gas production at manufacturing gas plants (MGP). CN was precipitated from the gas using iron oxides to form solid iron CN complexes. The waste products were often used as fill on the MGP site. The speciation of CN at MGP sites is dominated by the two solid complexes Prussian blue and Turnbull's blue. The complexes are stable at the low pH most often found in CN polluted soil at MGP sites, but the dissolution increases with increasing pH. By dissolution Prussian blue and Turnbull's blue release the two water soluble iron CN complexes ferro- and ferricyanide. According to equilibrium calculations ferro- and ferricyanide complexes are only stable at pH > 7.

Only at high pH levels in the soil are ferro- and ferricyanide complexes considered to constitute a human health risk. An increase of pH will increase leaching of the complexes to the groundwater and if the groundwater is exposed to sunlight before consumption, free CN can be formed. The risk of ingestion of CN polluted soil is difficult to predict since it will change from site to site depending on concentration, geology, climate etc. Both low and high risk evaluations can be found in the literature but in general it must be concluded to be site specific. Volatilization of free CN is only considered to constitute a risk in closed environments where the soil can be exposed to sunlight, e.g. greenhouses. Plant uptake of CN is not considered as an important exposure route for eatable crops growing above the soil surface, while CN concentrations in root vegetables growing in the soil, like carrots and radish, should be further investigated.

All vascular plants have an enzymatic system to metabolize CN formed during ethylene synthesis. Metabolism of artificially supplied CN was investigated with a phytotoxicity test and a metabolism test. Five different woody plants (willow, poplar, birch, elder and rose) were all able to remove free CN (HCN and CN^-) from a solution. The fastest removal was seen for willow trees (*Salix viminalis*) where removal rates for leaves and roots were around 9.5 and 7 mg/kg/h, respectively. Willow trees grown in a hydroponic solution containing free CN started to show phytotoxic effects at concentrations of 2 mg/L. When grown in sand the phytotoxicity was approximate a factor 10 lower. More than 90% of the free CN supplied to the willow trees was removed from the closed system (plant + flask) and no accumulation of CN was found in healthy plants. A non-linear mathematical model was used to balance estimates of uptake and metabolism. The model predicted that at low doses (< 10 mg/L when grown in sand), the CN would be rapidly metabolized. At higher doses, uptake would be faster than metabolism and consequently CN would accumulate in the plant tissue and toxic effects would occur.

When grown in hydroponic solutions containing ferro- or ferricyanide at concentrations up to 10 mg/L as CN, willow trees showed no phytotoxic effects. The speciation in the solutions became dominated by ferrocyanide during the experimental period, when an electron donor was present. It appeared that the removal of CN from the solution

decreased as the concentration of ferrocyanide increased, suggesting that uptake of ferricyanide is preferred over uptake of ferrocyanide. Increased concentrations of CN were found in all plant compartments (roots, stem and leaves) and between 10 and 50% of the CN were lost from the system. The highest percentage removal was found in the solutions with ferricyanide and with no additional source of nitrogen which implies that lack of nitrogen increases the uptake and decomposition of iron CN complexes.

The limiting factor for phytoremediation of CN polluted soil was concluded to be the dissolution of the solid iron CN complexes. An increase of soil pH, eventually by spreading of lime, is very likely to increase dissolution and plant uptake of iron CN complexes. In addition, an increase of soil pH can be necessary for the plants to survive in the typically very acidic soil. An increasing solubility of iron CN complexes also increases the possibility of leaching of CN to the groundwater; hence, the transpiration of the plants should be sufficient to prevent migration of water from the unsaturated zone to the groundwater.

Resumé

Cyanid (CN) var et biprodukt fra produktion af gas på gasværker. CN blev fjernet fra gassen ved udfældning med jern oxider i myremalm hvor de dannede et fast jern-CN kompleks. Den brugte myremalm blev ofte deponeret eller brugt som fyld på selve gasværksgrunden. Specieringen af CN på gasværksgrunde domineres af de to faste jern-CN komplekser Berliner blåt (Prussian blue) og Turnbulls blåt. Disse to komplekser er stabile ved lav pH, som ofte forekommer i CN forurenet jord på gasværksgrunde. Hvis pH forøges vil opløseligheden af de to komplekser stige og de to vandopløselige jern-CN komplekser ferro- og ferricyanid vil blive frigivet. Ifølge ligevægtsberegninger er ferro- og ferricyanid komplekserne kun stabile ved pH > 7.

Kun ved høje pH værdier i jorden kan ferro- og ferricyanid udgøre en risiko. En stigning i pH vil øge udvaskningen af komplekserne til grundvandet, og hvis grundvandet udsættes for sollys før det eventuelt drikkes, kan der dannes frit CN. Risikoen ved at indtage CN forurenet jord er svær at forudsige, da det vil være forskelligt fra sted til sted og afhænge af forhold som koncentrationer, geologi, klima m.m. Både lave og høje risikovurderinger er fundet i litteraturen, men generelt må det konkluderes at en risikovurdering er specifik for hvert sted. Afdampning af frit CN fra jorden vurderes til kun at udgøre en risiko i et lukket miljø, hvor jorden udsættes for sollys, f.eks. et drivhus. For frugt og grøntsager der vokser over jorden vurderes planteoptag af CN ikke til at udgøre nogen risiko, hvorimod CN optag og sorption til rodfrugter og grøntsager bør undersøges yderligere.

Alle karplanter har et enzymatisk system til at metabolisere CN dannet under ethylen syntese. Metabolisme af CN tilført planten udefra blev undersøgt vha. en fytotoksisitets test og en metabolisme test. Fem forskellige vedplanter (pil, poppel, birk, hyld og rose) var alle i stand til at fjerne frit CN (HCN og CN^-) fra en opløsning. Den hurtigste fjernelse blev fundet for piletræer (*Salix viminalis*) hvor blade og rødder var i stand til at fjerne CN med en hastighed på henholdsvis 9,5 og 7 mg/kg/time. Piletræer som voksede i en næringsopløsning indeholdende frit CN begyndte at vise tegn på en giftpåvirkning ved koncentrationer på 2 mg/L. Voksede piletræerne i sand var der først tegn på giftpåvirkning når koncentrationen var ca. en faktor 10 højere. > 90% af den frie CN i opløsningen blev fjernet fra det lukkede systemet (plante + flaske) og der blev ikke fundet akkumulering af CN i sunde planter. En ikke-lineær matematisk model blev brugt til at balancere estimater af optag og metabolisme. Modellen forudsagde at ved lav dosis (< 10 mg/L for planter der vokser i sand) vil CN blive hurtigt metaboliseret når det kommer in i planten. Ved højere dosis vil optaget af CN være større end metabolismen og der vil derfor ske en akkumulering af CN i planten, hvilket vil medføre giftpåvirkninger.

Hvis piletræer vokser i en næringsopløsning som indeholder ferro- eller ferricyanid vil koncentrationer op til 10 mg/L som CN ikke medføre nogen giftpåvirkning. Specieringen af CN i opløsningen vil under eksperimentets forløb blive domineret af ferrocyanid såfremt en elektrondonor er til stede. Det viste sig at fjernelsen af CN fra opløsningen

faldt samtidig med at koncentrationen af ferrocyanid steg, hvilket indikerer at planterne er mere villige til at optage ferricyanid end ferrocyanid. I alle plantedele (rødder, stamme og blade) blev der fundet forhøjede koncentrationer af CN og mellem 10 og 50% af den tilførte CN blev fjernet fra systemet. Den højeste procentvise fjernelse blev fundet i opløsninger med ferricyanid hvor der ikke var nogen yderligere kvælstofkilde, hvilket indikerer at kvælstofmangel kan forøge optag og dekomponering af jern-CN komplekser.

Det blev konkluderet at den begrænsende faktor for fytoremediering af CN forurenet jord sandsynligvis var opløseligheden af de faste jern-CN komplekser. En forøgelse af jordens pH værdi, evt. ved spredning af kalk, vil sandsynligvis forøge opløsning og planteoptag af jern-CN komplekser. Desuden kan det være nødvendigt at hæve jordens pH værdi for at planterne skal overleve i den typisk meget sure jord. En forøget opløsning af jern-CN komplekser vil også forøge muligheden for udvaskning af CN til grundvandet, og det er derfor nødvendigt at sikre sig at planterne transpirer nok vand til at forhindre nedsivning af vand fra den umættede zone til grundvandet.

1 Introduction

Cyanide (CN) is probably known to most people due to the intensive use of CN in crime stories. But CN also occurs naturally in several plants, bacteria, fungi and algae and is produced in large amounts for industrial purposes.

The use of CN is extensive and includes production of plastics, dyes, pigments, nylon, pharmaceuticals, insecticides, road salts, extraction of gold and silver ore and metal degreasing. The industrial use of CN has also made it an important environmental contaminant. The most important sources of anthropogenic CN contamination are metal mining discharges, iron and steel plants, electroplating facilities, former manufactured gas plants, organic chemical synthesis and road salt storage facilities (ATSDR, 2004). In this thesis the emphasis is put on CN pollution from gas production.

1.1 History of gas production

The history of gas production from coal goes back to the early 19th century when William Murdock started to heat coal in a closed iron vessel in his garden and used the gas for lighting his house. The use of gas for lighting soon became popular and the demand for gas increased.

The first Manufactured Gas Plant (MGP) in the UK was built in 1812 to lit the city of Westminster and within 10 years most of the countries larger towns and cities were lit by gas.

The first Danish manufactured gas plant opened in 1853 and in 1897, 51 MGP's were in operation in Denmark (Upton-Hansen et al., 1983). This was a result of the increasing demand of gas for lighting, heating and cooking, which the industrialization brought along.

The gas was produced from pyrolysis of coal and was a mixture of methane, carbon monoxide, hydrogen, and low carbon number illuminants. Undesired constituents like ammonia, hydrogen sulfide, CNs, and tars had to be removed before distribution of the gas. (Shifrin et al., 1995)

Cyanides and sulfur were removed in the last purification step. At first, this was accomplished using lime, but a new method using bog iron ore was patented in 1860. The gas was led through a box filled with bog iron ore. Removal of CN and hydrogen sulfur was a result of chemical reactions with the iron and can be described by the reactions (Buchwald, 1998):

$$2FeOOH + 3H_2S \leftrightarrow 2FeS + S + 4H_2O \tag{1.1}$$

$$14FeOOH + 36HCN + 3H_2S \leftrightarrow 2Fe_4[Fe(CN)_6]_3 + 3S + 28H_2O \tag{1.2}$$

The new method quickly gained popularity, as bog ore was much cheaper than lime and was often present in the underground. The use of bog ore was 8-21 kg/1000 m^3 gas produced (Theis et al., 1994). At the turn of the century the annual Danish consumption of bog ore for gas purification was around 1100 tons, while it in the 1940's was increased to 4-5000 tons (Buchwald, 1998; Christensen, 1966). A rough estimate of the total consumption of bog iron ore in Denmark is 250.000 tons.

The unawareness of the toxicity of the spent bog ore meant, that it was often deposited as fill on the MGP site, while in some places it was sold as pesticide or as raw material for sulfur production. The spent bog ore typically contained 1-2 % CN and 40-50 % sulfur. (Theis et. al., 1994)

As natural gas, which does not need the same purification, became more popular in the 1960's and 70's the MGP's were shut down. The last Danish MGP was closed in 1983 and today there are about 150 old abandoned MGP sites in Denmark, most of them being strongly polluted with CN, tar, PAH, BTEX and other hydrocarbons. USA and England have about 3000 MGP sites each, while there are approximately 1000 in Germany (Mansfeldt and Rennert, 2003).

To make distribution of the gas easier, the MGP's were often placed central in the towns. The central location means that it is often very attractive sites that are now polluted and useless. It also means that a large number of people might use the area on and around the site, and therefore are potentially exposed to the pollutants. Furthermore, the pollutants might threaten groundwater resources in the area. Waterworks are quite often found unsuitably close to the old MGP sites. This is, for example, the case of the two Danish MGP sites of Holte and Frederiksberg, where remediation of the sites has been necessary to prevent migration of contaminants to the groundwater.

Even though free CN is one of the most toxic compounds known, the remediation of MGP sites is usually altered by other pollutants like tar, PAH or BTEX. This is a result of CN being bound in metal complexes that are much less toxic than free CN.

The chemistry of CN is very complex, and the behavior of CN in soil is still not completely explained. The majority of the CN in the bog ore is complex bound with iron, often in the form of Prussian blue, $Fe_4[Fe(CN)_6]_3$, which has a very characteristic blue color. Other important CN compounds are thiocyanates, -SCN, and hexacyanoferrates, e.g. $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$.

Remediation of CN polluted soil usually involves removal and combustion or deposition of the soil. This method is not only very expensive but also not sustainable, and there is a large need for new and cheaper remediation technologies. Results from Trapp et al. (2001a+b) have shown that plants can take up CN but whether plants can remove CN from contaminated soil is not known.

1.2 Objectives

The objective of this study is to

i) make a risk assessment of CN polluted soil from a MGP site by evaluating the different exposure routes and the acute toxicity of the CN compounds in the soil.

ii) quantify uptake and metabolism of free CN in plants.

iii) establish a model that describes uptake, metabolism, accumulation and toxicity of CN in plants and validate the model with experimental data.

iv) investigate uptake and metabolism of complex CN in plants.

Table 2.1. Cyanide compounds in the environment.

Class	Name	Common names	Formula
Simple cyanides	Hydrogen cyanide	hydrocyanic acid Prussic acid	HCN
	Sodium cyanide		NaCN
	Potassium cyanide		KCN
	Cyanate	cyanic acid	HOCN
Iron complexed cyanides	Ferrocyanide	hexacyanoferrate(II)	$Fe(II)(CN)_6^{4-}$
	Ferricyanide	hexacyanoferrate(III)	$Fe(III)(CN)_6^{3-}$
	Ferri ferrocyanide	Prussian blue	$Fe(III)_4(Fe(II)(CN)_6)_3$
Thiocyanates	Thiocyanate	Rhodan hydrogen	HSCN

Box 2.1. Analysis methods for cyanide

Free, easily released, complex and total cyanide

The concentrations of free and total cyanide in soil, solution and plant material were analyzed according to the ISO 11262:2003 standard method. The method distinguishes between three different cyanide fractions:

- Easily released cyanide
- Complex cyanide
- Total cyanide

Easily released cyanide is the fraction that is liberated as HCN at pH 4 under reflux distillation for 1 hour. It is collected in a sodium hydroxide solution and analyzed photometrically. This fraction includes free cyanide, alkali salts and soft bound metal complexes.

Complex cyanide is the fraction that is left in the solution after liberation of the easily released fraction and consists mainly of iron and manganese complexes and some organic bound cyanide. The complex cyanide fraction is liberated as HCN using orthophosphoric acid under reflux distillation for 2 hours and is also collected in a sodium hydroxide solution and analyzed photometrically. The complex fraction contain iron, manganese and gold complexes and some organic forms (Smith and Mudder, 1991)

Total cyanide can be determined as the sum of easily released cyanide and complex cyanide or it can be measured as the complexed cyanide but without prior distillation of easily released cyanide.

Free cyanide (HCN and CN^-) is analyzed photometrically: HCN reacts with chloramine-T and forms cyanogen chloride, which will react with pyridine-4-carboxylic acid and 1,3-dimethylbarbituric acid to form a colored complex. The absorbance of the colored complex is measured at 606 nm, and subsequently it is possible to find the concentration of total cyanide from a standard curve.

The photometric determination of cyanide is, according to the ISO standard, applicable for solutions containing 0.1-1 mg/l. This corresponds to 0.5-5 mg/kg or mg/l when 10 g soil sample or 10 ml solution sample is tested.

According to Trapp et al. (2001a), the detection limit is 0.04 mg/kg or mg/l when a 10 g soil sample or 10 ml solution sample are tested. This corresponds to a concentration of 8 µg/l for the photometric determination.

Ferro- and ferricyanide

Quantification of ferro- and ferricyanide was conducted on a Perkin-Elmer LC 235, equipped with a C18 column (Phenomenex Luna 5 µm, 250 × 4.6 mm), a C18 pre-column (Phenomenex Security Guard), a Perkin-Elmer LC 235 diode array detector (λ:215 nm) and a Perkin-Elmer binary LC pump. All measurements were carried out at 35°C using a mobile-phase flow-rate of 1.0 mL/min and a sample volume of 50 µL.

The mobile phase used in the HPLC, a modification of the eluent used by Giroux and Barkley (1994), consisted of 35 % (v/v) acetonitrile and 65% Milli-Q water (Millipore Corporation, MA, USA) with 12.5 mM NaH_2PO_4 and 4 mM tetrabutylammonium hydrogensulfat ($C_{16}H_{36}NHSO_4$). NaH_2PO_4 and tetrabutylammonium hydrogensulfat was dissolved in the water phase which was filtered through a 0.45 µm cellulose nitrate filter before acetonitrile was added. The pH was adjusted to 8 with 1M NaOH and the solution was deaerated for 2-3 hours.

2 Cyanide in soil and groundwater at MGP sites

The fate of CN in the environment is difficult to predict due to the affinity of CN for making metal-CN complexes.

Studies by Meeussen et al. (1989, 1990, 1992a-b, 1994, 1995) investigated the chemistry of CN and focused on the solubility, transport, stability and decomposition of iron CN complexes in soils at former MGP sites. They determined different dissolution pathways and the solubility constant for Prussian blue. Ghosh et al. (1999a-c) also investigated the fate and transport of iron CN complexes in soil and water and modeled the speciation of CN under different redox and pH conditions. Theis et al. (1994) and Young and Theis (1991) determined the composition of CN polluted waste and leachate from MGP sites while Mansfeldt et al. (1998, 2000, 2001a-c, 2003) looked into different methods for determination of CN in various wastes and pH dependent leaching of CN from MGP soil. Rennert and Mansfeldt (2001a-b, 2002a-b) investigated different aspects of sorption of iron CN to soil and iron hydroxides.

2.1 Chemistry and speciation

Cyanide is a common name for compounds containing the cyanide group -C≡N. Cyanides can be divided into several groups, hydrogen cyanide being the most basic form. HCN is a liquid at room temperature with a boiling point of 25.5ºC and a characteristic smell of bitter almonds (ATSDR, 2004). Table 2.1 lists some of the most frequent cyanides in the environment while analysis methods for cyanides are described in Box 2.1.

On MGP sites, the majority of the CN in the soil is found as metal-CN complexes. 97 to 99% of the CN complexes involve iron (Gould et al. 1989; Theis et al., 1994). The dominating iron CN complexes are Prussian blue, $Fe_4[Fe(CN)_6]_3$, and Turnbull's blue, $Fe_3[Fe(CN)_6]_2$. The dissolution of Prussian blue was found to be a function of both pH and pe and the solubility product was determined to $K_{sp} = 10^{-66.5}$ by Meeussen et al. (1992a) and later found to $K_{sp} = 10^{-120.6}$ by Ghosh et al. (1999c) following the reaction

$$Fe_4(Fe(CN)_6)_{3\,(s)} \leftrightarrow 4Fe^{3+}_{(aq)} + 3Fe(CN)_6{}^{4-}{}_{(aq)} \qquad (2.1)$$

Under oxidizing conditions Fe^{3+} will precipitate releasing protons and thus further decrease pH of the soil

$$Fe^{3+}_{(aq)} + 3H_2O \leftrightarrow Fe(OH)_{3\,(s)} + 3H^+_{(aq)} \quad K = 10^{-3.2} \qquad (2.2)$$

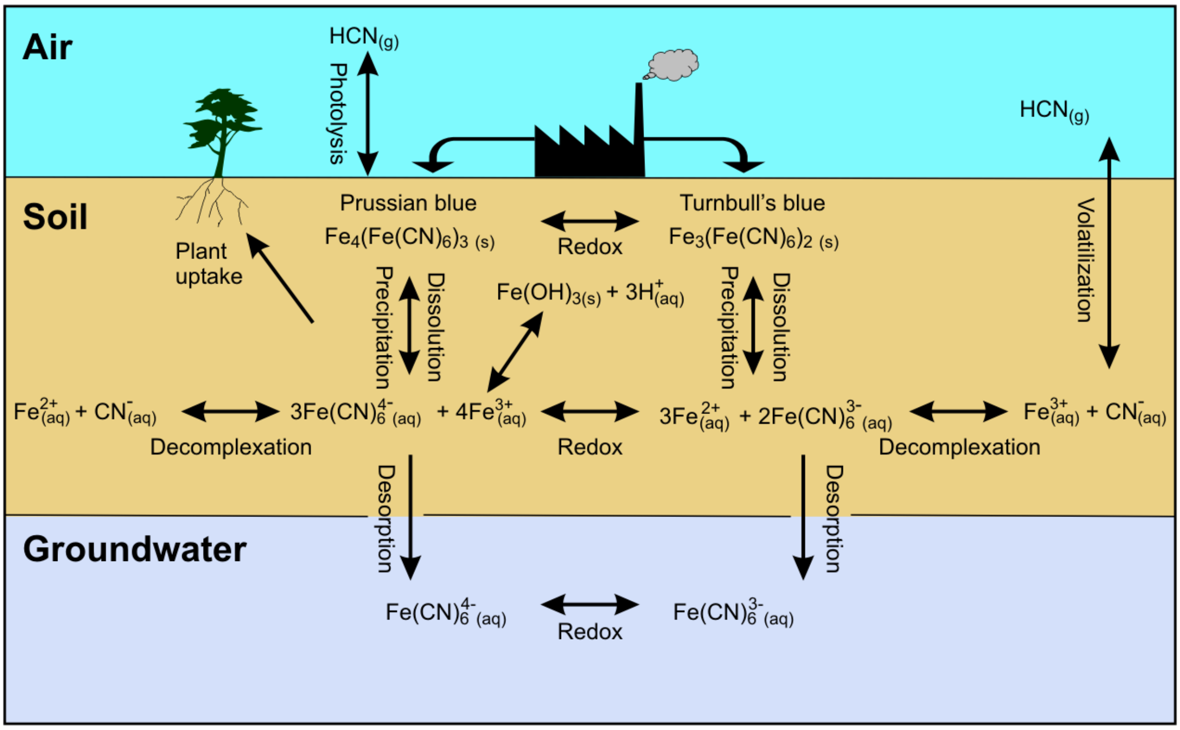
The solubility constant for Turnbull's blue was experimentally determined by Ghosh et al.(1999c) to $K_{sp} = 10^{-68.2}$ following the reaction

$$Fe_3(Fe(CN)_6)_{2\,(s)} \leftrightarrow 3Fe^{2+}_{(aq)} + 2Fe(CN)_6{}^{3-}{}_{(aq)} \qquad (2.3)$$

Table 2.2. Equilibrium constants for relevant processes in soil and groundwater at MGP sites. (Meeussen et al., 1992a; Ghosh et al., 1999c)

Reaction	LogK
$H^+ + CN^- \leftrightarrow HCN_{(aq)}$	9.2
$Fe^{3+} + e^- \leftrightarrow Fe^{2+}$	13.0
$Fe^{3+} + 6CN^- \leftrightarrow Fe(CN)_6{}^{3-}$	52.6
$Fe^{2+} + 6CN^- + e^- \leftrightarrow Fe(CN)_6{}^{4-}$	45.6
$Fe(CN)_6{}^{3-} + e^- \leftrightarrow Fe(CN)_6{}^{4-}$	-7.0
$Fe_4(Fe(CN)_6)_{3\,(s)} \leftrightarrow 4Fe^{3+} + 3Fe(CN)_6{}^{4-}$	-120.6
$Fe_3(Fe(CN)_6)_{2\,(s)} \leftrightarrow 3Fe^{2+} + 2Fe(CN)_6{}^{3-}$	-68.2

Box 2.2. Schematic overview of the controlling processes for cyanide at MGP sites.



The equilibrium between the ferro- and ferricyanide ions released by dissolution of Prussian blue and Turnbull's blue is described as

$$Fe(CN)_6^{3-} + e^- \leftrightarrow Fe(CN)_6^{4-} \qquad (2.4)$$

According to Meeussen et al. (1992a) is $K = 10^{-6}$ for the reaction, but using the newest stability constants (Ghosh et al., 1999c) the equilibrium constant changes to $K = 10^{-7}$. Thus, below pe = 7 iron CN complexes are mainly present in the reduced form, $Fe(CN)_6^{-4}$. Equilibrium constants for the most relevant processes in soil and groundwater are listed in Table 2.2.

Leaching of CN has been investigated in several studies, and they all concluded that the higher the pH, the higher the concentration of CN in the leachate (Theis et al., 1994; Ghosh et al., 1999c, 2004; Meeussen et al., 1990, 1992a-b, 1994, Kjeldsen, 1999). This can be explained by equation 2.2: An increase of pH will decrease the activity of Fe^{3+} and thus increase the dissolution of Prussian blue (equation 2.1).

Concentrations of total CN up to 63 g/kg soil have been reported (Mansfeldt et al., 2004), while concentrations of free CN are very low, often below the detection limit, due to the acidic conditions of the samples which would have permitted volatilization loss of HCN (Young and Theis, 1991; Theis et al., 1994; Ghosh et al., 2004). Box 2.2 gives a schematic overview of the controlling processes.

CN concentrations in groundwater below CN polluted MGP sites depend on the geology of the site. Not only dissolution but also sorption of the complexes is influenced by pH. Theis and West (1986) found that at pH 4, more than 95% of the ferrocyanide species in solution sorbed to goethite, whereas only 10% of ferrocyanide ions sorbed above pH 6.

The speciation of CN found by leaching experiments at various pH conditions was dominated by $Fe(CN)_6^{4-}$, which corresponds with the dissolution of Prussian blue (Theis et al., 1994).
Ghosh et at. (2004) measured speciation and total CN concentrations in groundwater samples collected from 10 different MGP sites. The concentrations ranged from 0.5 to 19.7 mg/L and the speciation of the CN complexes was dominated by the common $Fe(CN)_6^{4-}$ and a iron pentacyano complex with the possible stoichiometric formula of $[Fe(CN)_5NHCH_3]^{4-}$.

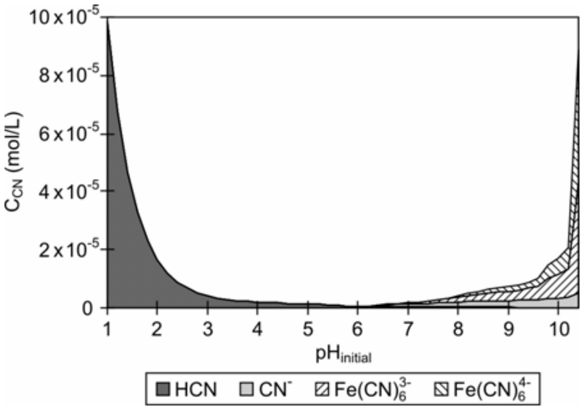


Figure 2.1. Equilibrium speciation of cyanide when 5 mg Prussian blue is dissolved at different initial pH values. The pe initial was 4. Calculated with PHREEQC.

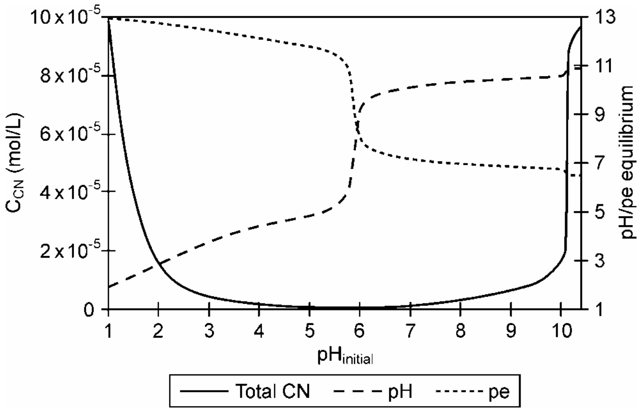


Figure 2.2. Change of pH, pe and total CN concentration when 5 mg Prussian blue is dissolved at different initial pH values. The pe initial was 4. Calculated with PHREEQC.

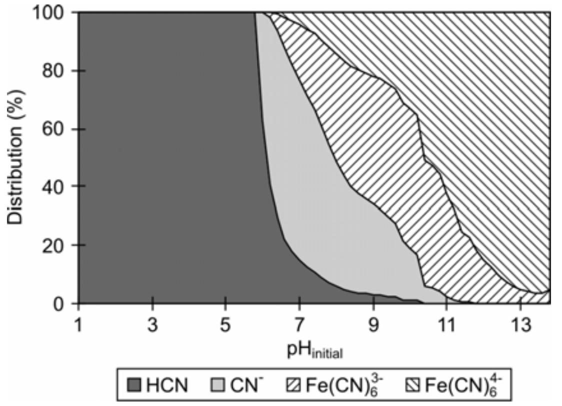


Figure 2.3. Distribution of the different cyanide species when 5 mg Prussian blue is dissolved at different initial pH values. The pe initial was 4. Calculated with PHREEQC.

2.2 Speciation modeling

Both Meeussen et al. (1992a+1994) and Ghosh et al. (1999c) have used chemical equilibrium programs to model speciation of CN in solution. Both found that HCN is the stable CN compound at low pH and that the solubility of Prussian blue strongly depends on pH and redox potential. Speciation calculations with the chemical equilibrium program PHREEQC (unpublished) confirm that HCN is the stable specie at pH < 6 when the dissolution of 5 mg/L Prussian blue is equilibrated over a range of initial pH values and an initial pE value of 4 (Figure 2.1). The concentration of total CN is lowest at an initial pH of 5.9 (equilibrium pH 7.9). At this point, the equilibrium pH and pe changes dramatically with small changes in initial pH as HCN start to dissociate to CN^- (Figure 2.2). At $pH_{initial} = 6.1$ ($pH_{equilibrium} = 9.2 = pK_a$) 50% of the CN is found as HCN and 50% as CN^- while ferro- and ferricyanide dominates at $pH_{initial} > 7.9$ ($pH_{equilibrium} = 10.1$) (Figure 2.3).

2.3 Risk assessment

Free cyanide, either as HCN or as CN^-, is the most toxic CN fraction. Free CN is quickly absorbed through inhalation, ingestion, or contact with the skin. It is then bound to an enzyme involved in respiration and thus inhibits the ability of cells to use oxygen (Shifrin et al., 1996). The lethal dose of free CN is estimated to be 0.5-3.5 mg/kg bodyweight, while the no-effect dosage is stated to 0.02 mg/kg bodyweight (ATSDR, 2004). Free CN is easily metabolized and therefore not accumulated in the body. Furthermore, it has never been verified that CN has any teratogenic, mutagenic or carcinogenic effects (Smith and Mudder, 1991).

Most other CNs are much less toxic. Thiocyanates have a lethal dosage of 50-80 mg/kg bodyweight, but chronic effects have been observed with a daily intake of 2-12 mg/kg bodyweight. For iron CN complexes dosages of up to 2 g/day of ferricyanide are not considered harmful (ATSDR, 2004).

The limit values for CN in soil and groundwater are, in Denmark, based on exposure to children. Aesthetic considerations are also taken into account, because iron CN complexes at very low concentrations color the soil strongly blue. Table 2.3 shows the Danish quality criteria for CN in soil, water, and air. Quite remarkably, no quality criteria have been set for CN in food. This is incomprehensible when the considerably high soil content of several vegetables is taken into account. Aside from this, CN may also be taken up and accumulated by some fruits and vegetables and the pesticides/fungicides/herbicide used on the crops can contain cyanide compounds (ATSDR, 2004).

Important exposure routes for CN at MGP sites could be:
- ingestion of soil and dust
- ingestion of crops from CN polluted soil
- dermal contact
- volatilization of free CN
- leaching to groundwater

Table 2.3. Danish quality criteria for cyanide (Danish EPA, 1995).

	Free	Easy released	Complex	Total	Thiocyanate
Soil (mg/kg)		10	500	500	500
Drinking water (mg/L)				0.005	
Recipient (mg/L)				1	
Indoor (mg/m^3)	5				

2.3.1 Ingestion of soil and dust

The used bog ore is usually placed in the uppermost soil layer, which gives a high exposure risk by ingestion or dermal contact. However, the CN is mainly present as iron complexes or as thiocyanate which have relatively low toxicity. The small quantities of free CN formed will usually escape from the soil by volatilization. Shifrin et al. (1996) concluded from a risk analysis that there is very little concern connected with ingestion or dermal contact. The question is whether Shifrin et al.'s risk analysis is as conservative as they claim. They use a daily ingestion of 200 mg soil, which is in the lower end of the typically used interval between 0.1 and 2 g (Danish EPA, 1990). Besides this, pica children are not taken into consideration. Pica children are children with a morbid propensity to ingest soil. These children are capable of ingesting 3-6 g of soil pr day, and in special cases a single dose of 20-30 g of soil. Another ingestion route that Shifrin et al. do not take into consideration is the ingestion of soil in vegetables. Vegetables like leeks, carrots, lettuce and cabbage can contain considerable amounts of soil, which should also be taken into account in the analysis.

Trapp and Christiansen (2003) measured easily liberatable CN concentrations up to 270 mg CN/kg soil and concluded that the risk of CN poisoning by direct soil uptake is very high. The conclusion was based on results from one specific site and can not be generalized

Due to the phytotoxicity of used bog ore, an old abandoned MGP site will often lay bare without any vegetation. This increases the formation of dust from the soil. Dust can enter the human body by both ingestion and breathing, and is a possible pathway for uptake of pollutants. However, breathing of dust is, in most cases, not as important an exposure route as ingestion or dermal contact (Danish EPA, 1990). Cases where breathing of dust can be relevant are in arid areas or if the soil consists of very small particles or is exposed to wind.

2.3.2 Ingestion of crops from cyanide polluted soil

Experimental data from Larsen et al. (2004+2005), Trapp et al. (2001a+b) and Ebbs et al. (2003) have shown that transport and allocation of both easily released and complex CN is possible. Trapp et al. (2001b) found concentrations of easily liberatable CN in willow roots up to 900 mg/kg when grown in CN polluted MGP soil. It was not determined whether the CN was sorbed or inside the roots. Willows grown in a hydroponic solution with ferricyanide, ferrocyanide or Prussian blue transported and allocated CN as well, although in much lower concentrations (Larsen and Trapp, 2005, Trapp et al., 2001b). Thus, it seems as CN concentrations in leaves and fruits is of no concern, while caution should be taken with root vegetables e.g. carrot and radish.

2.3.3 Dermal contact

Dermal contact with CN polluted soil is not considered to be a health risk. The CN will be bound in iron and thiocyanate complexes with very low toxicity. Skin irritation can occur at used bog iron ore concentration above 5%. This is probably due to the low pH or the tar content and not due to the CN complexes (Danish EPA, 1990).

2.3.4 Volatilization of free cyanide

A small quantity of free CN will be formed due to equilibrium with iron-complexed CN. The free CN formed will, due to the relatively high Henry's constant of CN, escape by volatilization. Inhalation is a potential exposure route to the volatilized CN. Both Shifrin et al. (1996) and Environmental Resources Ltd. (1987) concluded that volatilization of CN from old MGP sites is not expected to be an important route of exposure. None of the analyses considered formation of free CN from soils exposed to sunlight. Sunlight only reaches the uppermost thin soil layer, and the free CN will be strongly diluted in the air.

Larsen and Trapp (2003) measured volatilization from CN polluted MGP soil under different conditions (Table 2.4). Laboratory results with 2 different soils showed that volatilization was significantly lower when the soil was kept dark compared to when the soil was exposed to light (Figure 2.4). When the pH of the soil was increased by addition of CaO the volatilization decreased. *On site* measurements showed that CN was only detectable when a passive sampler was placed in a closed container positioned directly on the soil. They concluded that volatilization of CN should only be considered in a closed environment, e.g. a greenhouse.

Table 2.4. Characteristics of soils used in volatilization experiments.

Condition		Soil 1	Soil 2
Initial	pH	2.1 ± 0.1	1.9 ± 0.1
	moisture content	11.6 ± 0.4%	29.4 ± 2.2%
Soil dried, CaO+water added	pH	5.2 ± 0.2	6.3 ± 0.2
	moisture content	25%	25%

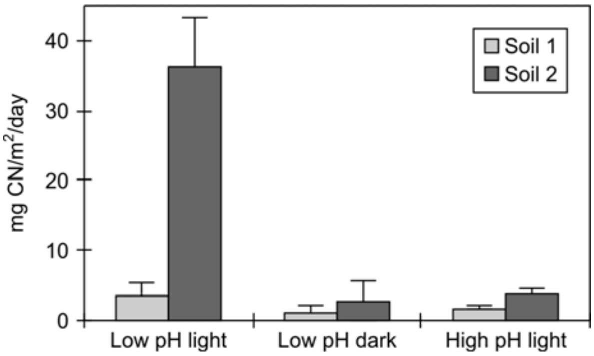


Figure 2.4. Volatilization of free cyanide from 2 different cyanide polluted MGP soils under different conditions.

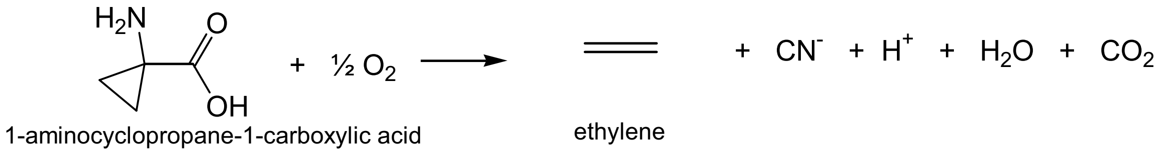
2.3.5 Leaching to groundwater

The low pH in used bog ore results in low leaching of iron CN complexes. For that reason, high concentrations of CN in the pore or groundwater can only be expected if the ore has been mixed with alkaline soils, or if lime has been spread on the soil. After a period of time, the infiltration of rainwater will also increase the pH, and a higher leaching can be expected. A dangerous scenario can be, if the groundwater polluted with iron CNs is used for drinking water and stored in the sun, which may lead to exposure to free CN.

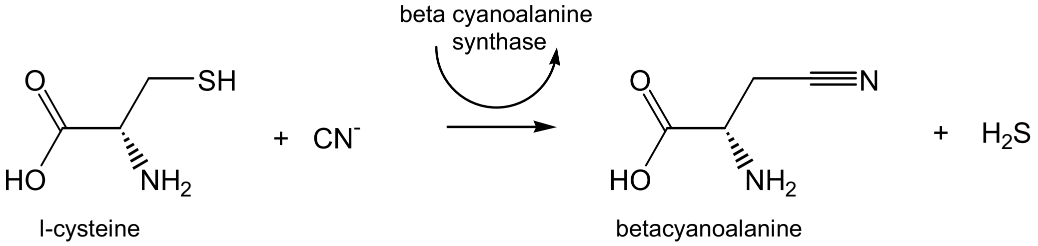
According to Kjeldsen (1999), there is an existing risk of groundwater exposure to iron-complexed CN. He used an example with groundwater containing iron-complexed CN with a realistic concentration of 2-12 mg CN/l, and showed that if it is all decomposed, a 15 kg child consuming 1 liter of water would get a dose of 0.8 mg/kg/day, which is close to the fatal dose.

Box 3.1. Formation and metabolism of cyanide in vascular plants.

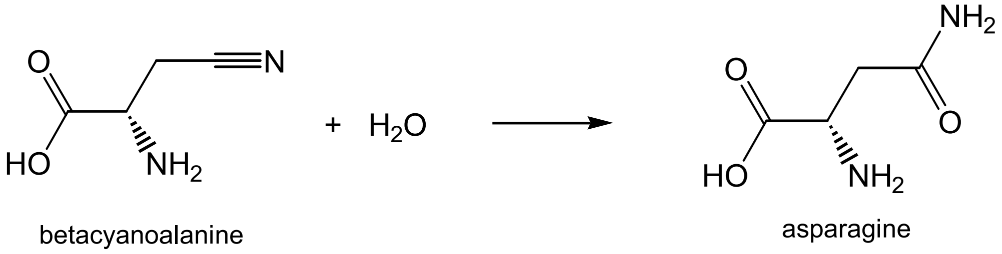
Cyanide is formed during the conversion of ACC (1-aminocyclopropane-1-carboxylic acid) to ethylene (Parlar and Angerhöfer, 1995):



The most common detoxifying reaction is where cyanide reacts with L-cysteine to form β-cyanoalanine under the influence of the enzyme β-cyanoalanine synthase:



β-cyanoalanine can afterwards be hydrolyzed to asparagine:



Another, but far less common enzyme that can metabolize cyanide in higher plants is rhodanese (thiosulfate-thiotransferase) (Miller and Conn, 1980). This enzyme can convert cyanide into thiocyanate, -SCN, which is much less toxic.

$$CN^- + S_2O_3^{2-} \xrightarrow{\text{rhodanese}} SCN^- + SO_3^{2-}$$

3 Uptake, metabolism, accumulation and toxicity of free cyanide in plants

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All vascular plants are capable of producing CN and more than 2000 cyanogenic plants are known (Miller and Conn, 1980). CN can be produced as a result of degradation of cyanogenic glucosides. This was expected to be the main contributor to CN in plants until Peiser et al. (1984) and Pirrung (1985) demonstrated that CN is formed as a co-product during ethylene synthesis (see Box 3.1). Ethylene is a plant hormone, which is involved in several processes such as germination, senescence and ripening.

Due to the phytotoxicity of CN, it is necessary for the plant to detoxify the CN formed. This is done by enzymatic reactions and the most common enzyme to detoxify CN is β-cyanoalanine synthase. Miller and Conn (1980) found β-cyanoalanine synthase in every higher plant they tested, both cyanogenic and non-cyanogenic, while other enzymes catalyzing metabolism of CN (rhodanese and FHL) occurred far less commonly in plants.

As early as in 1968 Blumenthal et al. suggested that the conversion of HCN to asparagine could be a detoxification mechanism. Castric et al. found in 1972 that “when $H^{14}CN$ is administered to young sorghum seedlings, the radioactivity is quickly and extensively transferred to the amide group of asparagine”. They also tested other plants, both cyanogenic and non-cyanogenic, and found asparagine and nothing else, although it was found that the conversion was not as effective in the non-cyanogenic plants. Miller and Conn (1980) also found that all plants they tested, both cyanogenic and non-cyanogenic, were capable of metabolizing HCN.

Peiser et al. (1984) concluded that “since ethylene formation occurs widely in all plants and is accompanied by CN formation, it is necessary that all plants have the enzyme(s) to metabolize CN”. They also found, when comparing plants ability to metabolize CN with the production of CN due to ethylene synthesis, that the plants had ample capacity to remove the CN formed. The β-cyanoalanine synthase activity from 15 plants ranged from 3.9 to 1050 nm/g/min (6 to 1638 mg CN/kg/h), while the ethylene production rate ranged from 0 to 0.2 nmol/g/min (0 to 0.3 mg CN/kg/h).
According to Grossmann (1996), the capacity to metabolize CN depends on the age of the plant tissue. The capacity should be greater in older plant tissues and areas with meristematic activity.

Hendrickson and Conn (1969) found during their work with CN metabolism that β-cyanoalanine synthase has a pH optimum of about 9.5 and a molecular weight of about 53000 g/mol. They also found the K_M values for L-cysteine and CN to be 2.5 mM and

0.55 mM (14.3 mg CN/l), respectively, in the reaction forming β-cyanoalanine, and stated that the reverse reaction does not occur under conditions of the forward reaction. According to Hendrickson and Conn, the β-cyanoalanine synthase requires no added co-factors.

3.1 Plant uptake

Since many plants are capable of producing and degrading CN it has also been investigated whether plants can degrade CN supplied from an artificial source. Larsen et al. (2004) demonstrated that 5 different woody plants (willow, poplar, birch, elder and rose) all were able to remove free CN (HCN and CN^-) from a solution. When willow trees were grown in solutions with concentrations of 0.4, 2, 8 and 20 mg/L, total CN in most solutions had decreased to levels below the detection limit (0.04 mg/L) at the end of the experiment (192 h). Significant increase of CN inside the plant was only found in willow trees suffering from the toxicity of CN (2, 4 and 8 mg/L), while no CN was accumulated in the trees exposed to 0.4 mg/L. The same pattern was seen for willow trees grown in sand and irrigated with CN solutions at different concentrations (Larsen et al., 2005). 89-98% of the CN supplied was removed from the system and accumulation in the trees was only seen at the highest concentrations (40-50 mg/L) where the trees were dying (Table 3.1). Volatilization, sorption and degradation were found to account for a loss of 30% in the controls.

Results from Trapp et al. (2001b) were very similar. They found that less than 1% of the CN could be recovered from the solution, while about 1% of the supplied CN could be found in the plants, when willow trees (*Salix viminalis*) were grown in a CN solution at different concentrations. Again, accumulation inside the plant was only found for toxic levels of CN. They measured volatilization from the system by placing the flasks with trees in a desiccator and found that 1% of the CN was lost due to volatilization.

Ebbs et al. (2003) used a different willow species (*Salix eriocephala* L. var. Michaux) and used ^{15}N labelled CN to quantify transport and metabolism. They found that 94% of the CN was removed from a solution with an initial free CN concentration of 2 mg/L after 20 days. Despite a significant enrichment in ^{15}N content CN concentrations in the plant tissue was not significant different from control values. A mass balance based on the ^{15}N content of the plant tissue recovered 60% of the CN.

Table 3.1. Final cyanide mass balance for willow trees grown in sand and irrigated with a solution containing cyanide at different concentrations. In brackets: Standard deviation.

C_{added} (mg/L)	Mass of CN (µg)						Loss (%)
	Sol, initial	*Root*	*Stem*	*Leave*	*Sol, final*	*tot*	
0	0 (0)	0.4 (0.3)	0.5 (0.4)	0.3 (0.3)	30.8 (10.5)	32	
10	1019 (34)	2.0 (3.0)	1.3 (1.2)	1.2 (0.9)	35.8 (6.0)	40.3	96
20	1941 (44)	1.4 (0.5)	0.9 (1.8)	0.8 (0.6)	52.6 (14.2)	55.7	97.1
30	2987 (23)	1.8 (0.8)	1.6 (2.0)	1.5 (0.3)	50.6 (33.2)	55.5	98.1
40	3977 (22)	23.6 (10.6)	40.5 (-)	1.6 (1.8)	300.7 (200.2)	366.4	90.8
50	4991 (17)	35.2 (21.9)	23.7 (30.3)	1.1 (0.6)	483.4 (173.4)	543.4	89.1

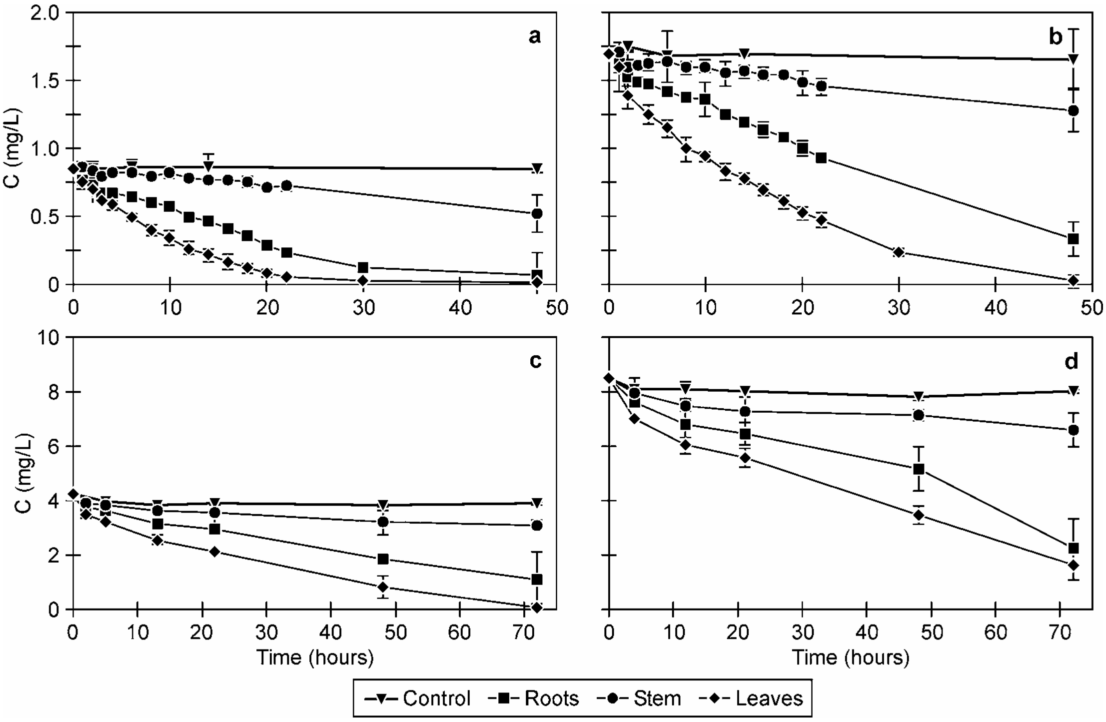

Figure 3.1. Decrease of cyanide concentration in the closed-bottle metabolism test. Error bars denote the 95% C.I.

Box 3.2. The closed-bottle metabolism test

The purpose of the closed-bottle metabolism test is to determine the metabolism/removal rates of a chemical for roots, stem tissue and leaves of a plant.

Roots and leaves are cut off the stem. The stem is cut into slices not thicker than 2 mm. Roots, stem slices and leaves are dried with a paper tissue, weighed and transferred to 250 mL gas-dense blue-cap bottles. A solution (200 mL, pH 7) containing the chemical at different concentrations is added to the blue-cap bottles containing the plant material (2 g). The bottles are placed on a shaking bench at 75 rpm to ensure mixing. The concentration of the chemical in solution is measured at relevant intervals. The experiments are stopped when the concentration of the chemical approaches zero, or when no further removal is measured. The total concentration of the chemical in the plant material is determined.

From the degradation rates the maximum degradation rates, v_{max}, and the *Michaelis-Menten* constants, K_M, can be determined by non-linear fit.

Table 3.2. Michaelis-Menten parameters obtained by non-linear regression. ± 95% C.I.

	v_{max} (mg/kg/h)	K_M (mg/L)	R^2	n
Roots	6.9 ± 1.7	0.44 ± 0.33	0.29	55
Stem	1.6 ± 0.7	0.01 ± 0.51	1.5×10^{-05}	23
Leaves	9.6 ± 1.1	0.59 ± 0.20	0.73	84

3.2 Kinetics

Enzymatic reactions can be described by Michaelis-Menten kinetics

$$v = \frac{v_{\max} C}{K_M + C}$$

where v is the disappearance velocity (mass per time) of the substrate of concentration C, v_{max} is the maximum disappearance velocity, and K_M is the half-saturation constant (the substrate concentration where the reaction velocity is half the maximum). Larsen et al. (2004+2005) used a closed-bottle metabolism test (see Box 3.2) to determine metabolism rates of CN for roots, stem and leaves of willow (*Salix viminalis*). They found that all parts of the plant were able to metabolize CN but at different rates (Fig. 3.1). Leaves showed the fastest removal and stem the slowest (Table 3.2). The rates varied with dosage. The removal half-time was lower at low doses, although the absolute transpiration was higher at high doses.

Yu et al. (2004) used a similar test method for leaves of 28 different Chinese plant species and found CN removal capacities between 0.60 and 8.77 mg/kg/h while Trapp et al. (2003) found removal rates for leaves and roots of sorghum (*Sorghum bicolor*) of 3.1-4.7 mg/kg/h and 2.8-6.9 mg/kg/h, respectively.

Box 3.3. Principle of the phytotoxicity test.

The phytotoxicity test was designed by Trapp et al. (2000) and uses growth and transpiration as parameters for evaluation of short-term acute toxicity of compounds in solution or soil to trees.

Willow trees are pre-grown 2-4 weeks before they are transferred to 500 mL Erlenmeyer flasks with about 400 mL nutrient solution. The flasks are sealed with cork stoppers and covered with aluminum foil to prevent volatilization and algae growth, and placed in a climate chamber with artificial light and constant temperature and humidity. The initial loss of weight is measured after 24 h. The plants are then transferred into flasks with soil or a spiked solution while controls are transferred into unpolluted soil or remained in the nutrient solution. The weight is measured every 24 h during the experimental period, typically 72-144 h. The normalized relative transpiration is used to compare the toxic effect on trees with different initial transpiration and calculated as:

$$NRT(C,t)=\frac{\frac{1}{n}\cdot\sum_{i=1}^{n}T_i(C,t)/T_i(C,0)}{\frac{1}{m}\cdot\sum_{j=1}^{m}T_j(0,t)/T_j(0,0)}$$

where C is concentration (mg/L), t is time period (h), T is absolute transpiration (g/h), i is replicate 1, 2, …, n and j is control 1, 2, …, m.

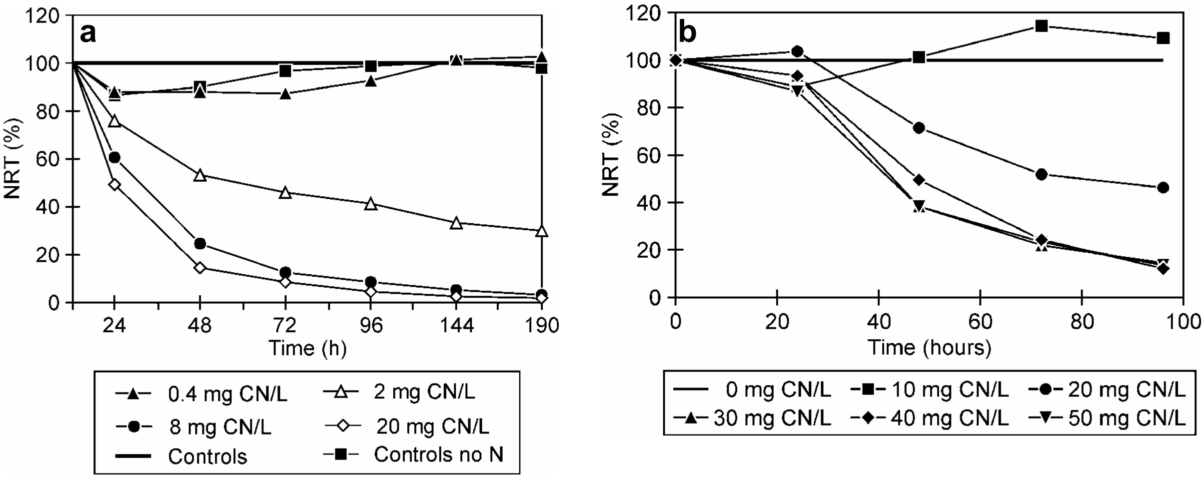

Figure 3.2. Normalized relative transpiration (NRT) of willows grown in (a) hydroponic solution and (b) sand supplied with cyanide solution at different concentrations.

3.3 Phytotoxicity

The phytotoxicity of CN must be taken into account if plants are considered for removal of CN from soil, surface- or groundwater. Larsen et al. (2004 and 2005) measured phytotoxicity of free CN to willow trees (*Salix viminalis*) with a recently developed phytotoxicity test (Trapp et al., 2000), which uses the transpiration of plants as parameter for toxicity (see Box 3.3). The results showed a significant difference between trees grown in solution and trees grown in sand. When grown in solution a concentration of 2 mg/L decreased the normalized relative transpiration (NRT) with more than 50% after 72 h while the trees were dead after 3 weeks. Trees exposed to 8 and 20 mg/L quickly showed signs of wilting and were dead after < 1 week (Fig. 3.2a). When grown in sand and irrigated with a solution containing CN the phytotoxicity was approximately a factor 10 lower (Fig. 3.2b). 20 mg/L of CN in the irrigation water decreased the NRT with > 50% after 96 hours while concentrations of 30, 40 and 50 mg/L killed the plants. The difference in phytotoxicity was explained by a slower uptake of CN when trees were grown in sand.

Trapp & Christiansen (2003) found that willows (*Salix viminalis*) exposed to 5 mg/L in solution decreased the NRT with 50% after 96 h and were dead after 3 weeks.
Experiments from Ebbs et al. (2003) using a different willow species (*Salix eriocephala* L. var. Michaux) showed no effect on the transpiration when grown in a CN solution of 2 mg/L. Sorghum (*Sorghum bicolor*) grown in soil and irrigated with solutions of 20 and 50 mg CN/L revealed no signs of toxicity but increased the transpiration compared to controls (Trapp et al., 2003). The experiments with sorghum were not performed in a closed environment, thus volatilization probably plays an important role and the results are not directly comparable to the results from the willow trees.

Box 3.4. Non-linear model to calculate uptake, metabolism, accumulation and toxicity of cyanide in willow trees (Larsen et al., 2005).

The mass balance for cyanide in roots can be divided into

(1) Inflow via transpiration: $C_W \times Q$

(2) Outflow via xylem: $-C_{Xy} \times Q$

assuming equilibrium between translocation stream and root tissue: $C_{Xy} = \dfrac{C_R}{K_{RW}}$

(3) Uptake by diffusion: $A \times P \times \left(C_W - \dfrac{C_R}{K_{RW}} \right)$

(4) Growth: $-k_R \times C_R$

(5) Metabolism: $-\dfrac{v_{\max} \times C_R}{K_M^* + C_R} \times M_R$

C_W = concentration in water (mg/L)
Q = transpiration (L/d)
C_{Xy} = concentration in xylem (mg/L)
C_R = concentration in roots (mg/kg)
K_{RW} = partition coefficient root tissue vs transpiration stream (L/kg)
k_R = growth rate of roots (d^{-1})
v_{max} = maximal removal velocity (mg/kg/d)
K^*_M = half-saturation constant (mg/kg)
M_R = mass of roots (kg)
A = surface area of roots (dm^2)
P = permeability of roots (dm/d)
m_R = mass of cyanide in root (mg)

The complete mass balance is then

$$\frac{dm_R}{dt} = (Q + A \times P) \times C_W - (Q + A \times P) \times \frac{C_R}{K_{RW}} - \frac{v_{\max} \times C_R}{K_M^* + C_R} \times M_R$$

or

$$\frac{dC_R}{dt} = \left(\frac{Q + A \times P}{M_R} \right) \times C_W - \left(\frac{Q + A \times P}{M_R \times K_{RW}} + k_R \right) \times C_R - \frac{v_{\max} \times C_R}{K_M^* + C_R}$$

At steady state (*$dC_R/dt = 0$*) the solution of the model is a quadratic equation which can easily be implemented in a spreadsheet and solved.
A similar mass balance was written for stem and leaves. Diffusive uptake in/out of the stem was neglected because the process could not be quantified. Exchange with air from the leaves was considered by adding terms for uptake and loss to air.

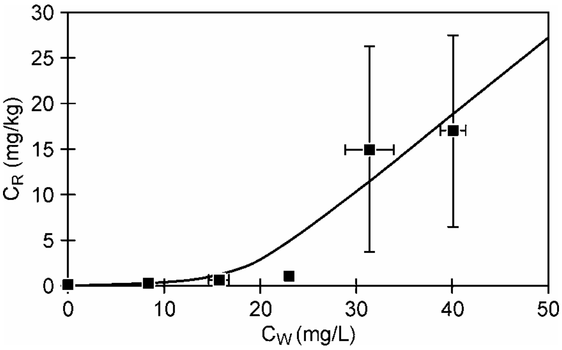

Figure 3.3. Measured and simulated cyanide concentrations inside the roots (*C_R*) for varying concentrations in the external solution (*C_W*). Error bars denote 95% C.I.

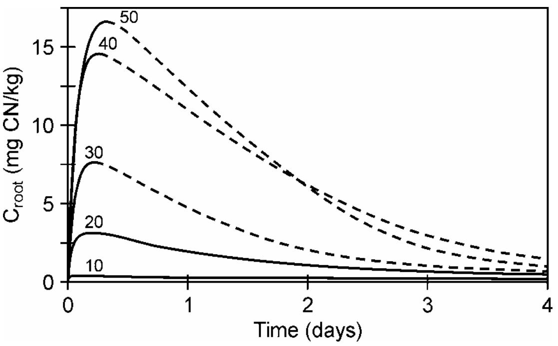

Figure 3.4. Simulation of cyanide concentrations inside the roots (C_R) for different concentrations in the external solution. The lines are dotted for the trees that died.

3.4 Modeling

A non-linear mathematical model to determine uptake, metabolism and toxicity of free CN in willow trees was developed by Larsen et al. (2005). The model uses diffusive and advective uptake through the roots and translocation via the xylem sap to calculate concentrations of CN in roots, stem and leaves (Box 3.4). Parameters used in the model were either experimentally determined or found in the literature.

The simulation of the concentration inside the roots (C_R) for increasing CN concentrations in the external solution (C_W) showed that metabolism inside the roots was as fast as the uptake and no accumulation occurred for C_W below 10 mg/L, thus no toxic effect would be expected (Fig. 3.3). At higher concentrations v_{max} is reached but the uptake still increases, thus C_R increases linear with C_W. Measured concentrations were also plotted and showed a similar trend.

A sensitivity analysis on the model found that the sensitive parameters for simulation of the CN concentration in roots were root mass (40.2%), v_{max} (39.7%), root surface area (11.6%) and transpiration (8.3%).

Simulations with parameters from specific experiments were carried out to compare simulated and measured values (Fig 3.4). When CN at 10, 20 and 30 mg/L was applied, good agreement between simulated and measured concentrations of CN in the roots was found while at higher concentrations the simulated values were much lower than the measured, though the maximal simulated concentrations over the experimental period were very close to the experimental (Table 3.3). A likely reason is that when the plants suffer due to toxic effects, the metabolic capacity is decreased.

Table 3.3. Comparison of experimental and simulated concentrations (mg/L) of cyanide in the plants at 4 Days.

C	$C_{Experimental}$			$C_{Modeled, final}$			$C_{Modeled, max}$		
	root	stem	leaves	root	stem	leaves	root	stem	leaves
0	0.24	0.04	0.12						
10	0.23	0.06	0.26	0.20	$3.9\text{x}10^{-4}$	$4.1\text{x}10^{-7}$	0.36	$5.0\text{x}10^{-5}$	$4.2\text{x}10^{-7}$
20	0.72	0.07	0.24	1.08	$1.3\text{x}10^{-4}$	$8.5\text{x}10^{-7}$	3.12	$6.3\text{x}10^{-4}$	$6.8\text{x}10^{-6}$
30	1.09	0.12	0.51	2.04	$1.5\text{x}10^{-4}$	$7.8\text{x}10^{-7}$	7.65	$1.8\text{x}10^{-3}$	$2.7\text{x}10^{-5}$
40	15.00	2.26	0.63	6.18	$3.8\text{x}10^{-4}$	$1.6\text{x}10^{-6}$	14.58	$3.4\text{x}10^{-3}$	$4.5\text{x}10^{-5}$
50	17.03	1.87	0.62	6.02	$4.0\text{x}10^{-4}$	$1.8\text{x}10^{-6}$	16.58	$4.0\text{x}10^{-3}$	$5.1\text{x}10^{-5}$

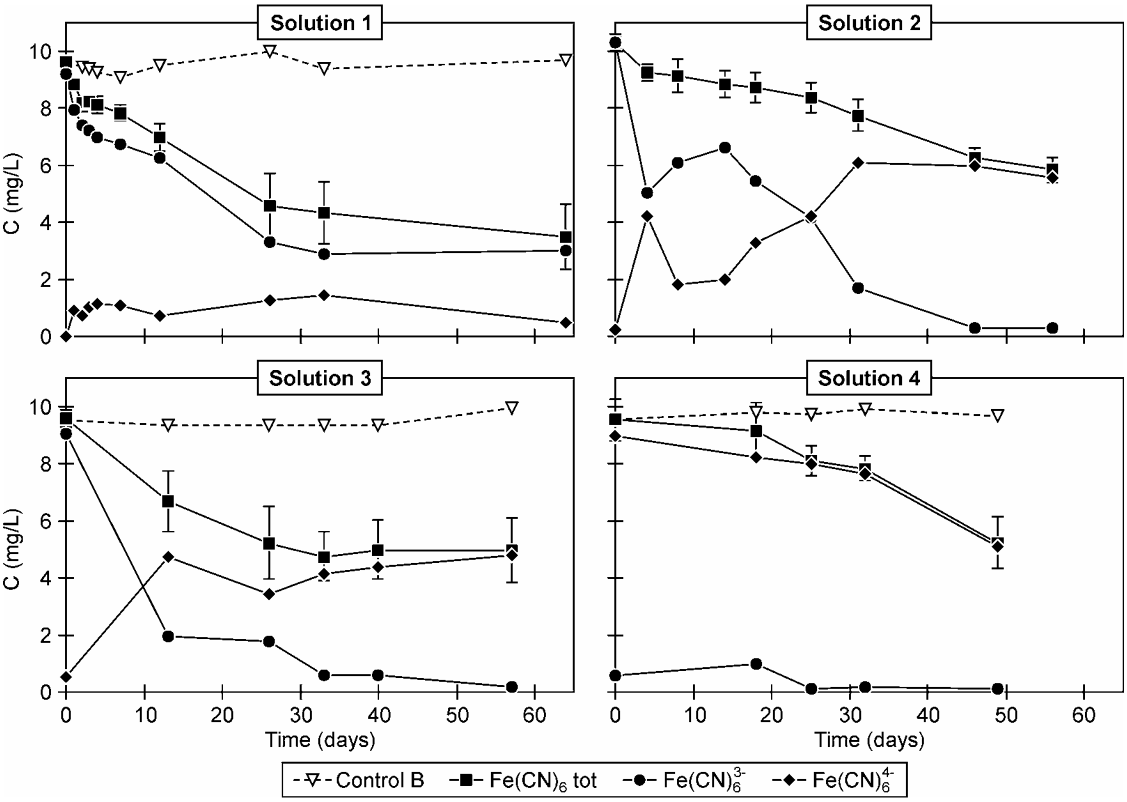

Figure 4.1. Concentration and speciation of cyanide in 4 different solutions: (1) Distilled water with ferricyanide; (2) Nutrient solution with ferricyanide as the only iron source; (3) Nutrient solution with ferricyanide as the only iron and nitrogen source; (4) Nutrient solution with ferrocyanide as the only iron and nitrogen source. Initial concentrations of 10 mg/L as CN. Error bars denote 95% C.I.

4 Uptake of iron cyanide complexes into willow trees

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Plant uptake and metabolism of free CN has been proven (see chapter 3) but since the main part of CN found in soil and groundwater is complex bound with metals, uptake, transport and metabolism of metal cyanide complexes is a decisive factor for successful remediation of CN polluted soil with plants.

Transport of organic compounds and metal-chelate complexes in plants has been shown (Castro et al., 2003; Burken and Schnoor, 1997; Epstein et al., 1999) and in view of the fact that Beavis and Vercesi (1992) showed that a mitochondrial inner membrane anion channel can transport ferrocyanide, there is a reason to believe that metal-cyanide complexes can be taken up and metabolized.

As described in chapter 2, the speciation of CN is influenced by several factors. At MGP sites the speciation is dominated by the 2 solid complexes Prussian blue and Turnbull's blue which are releasing ferro- and ferricyanide when dissolved.

4.1 Phytotoxicity

Compared to free CN the plants can tolerate much higher concentrations of metal cyanide complexes before any phytotoxic effects are seen. Larsen and Trapp (2005) saw no phytotoxic effects on willow trees (*Salix viminalis)* exposed to ferri- or ferrocyanide at 10 mg/L as CN. Trapp and Christiansen (2003) grew balsam poplars (*Populus trichocarpa*) in solutions containing Prussian blue. At concentrations of 10 mg/L (5.4 mg/L as CN) no phytotoxic effects was observed while concentrations of 50 mg/L (27 mg/L as CN) decreased the normalized relative transpiration slightly. Even at 2500 mg/L (1350 mg/L as CN) the trees survived though the transpiration was reduced with 80%.

4.2 Concentration and speciation

As a rule Fe(II) is taken up into plants preferentially compared with Fe(III), but it also depends on the plant species. Most of the iron in plants is in the ferric (Fe(III)) form and long-distance transport of iron in the xylem is dominated of Fe(III) complexes. It is unlikely that ionic Fe^{3+} and Fe^{2+} are of any importance in short- or long-distance transport in plants (Marschner, 1995). Larsen and Trapp (2005) investigated uptake of ferro- and ferricyanide in willow trees (*Salix viminalis*). They used 5 different solutions and found that in solutions with ferricyanide in distilled water (solution 1) the speciation remained almost stable (Figure 4.1) while in solutions with ferricyanide in nutrient solution the speciation became dominated by ferrocyanide during the experimental period (solution 2 and 3). Nutrient solutions initially supplied with ferrocyanide (solution 4) maintained the speciation throughout the experimental period. In the controls without trees the speciation was stable over the experimental period for all solutions. Ferricyanide can be reduced extracellularly by the ferrireductase to ferrocyanide. The formation of ferrocyanide in solutions 2, 3 and 5 with trees would thus be expected as a result of ferrireductase. The reduction of ferricyanide did not occur in experiments with distilled water (solution 1).

The concentration of iron cyanide decreased over time in all the experiments, but at different rates. The fastest loss was seen for the solutions initially containing ferricyanide and no other nitrogen source. It appears that the removal from the solution decreases as the concentration of ferrocyanide increases suggesting that uptake of ferricyanide is preferred over uptake of ferrocyanide. Also nitrogen seems to play a role for the uptake. The loss in solution 2, which had nitrate in the nutrient solution, was significantly lower than the loss in solutions 1 and 3 (no nitrate), which implies that the lack of nitrogen in solutions 1 and 3 increased the uptake and decomposition of iron cyanide.

A part of the loss from the solution can probably be explained by sorption to the roots. A sorption study with root cell wall skeletons (2 g/L FW) from willow trees (*Salix eriocephala* L. var. Michaux) in a ferrocyanide solution of 2 mg/L as CN showed that 23% of the iron cyanide sorbed to the cell wall (Ebbs et al., 2003).

Table 4.1. Percentage distribution of cyanide in plants and solution for 5 different solutions: (1) Distilled water with ferricyanide; (2) Nutrient solution with ferricyanide as the only source of iron; (3) Nutrient solution with ferricyanide as the only source of iron and nitrogen; (4) Nutrient solution with ferrocyanide as the only source of iron and nitrogen; (5) Nutrient solution with Prussian blue as the only source of iron and nitrogen. In brackets: Standard deviation.

Sol	C (mg/L)	Distribution (%)				
	initial	*Root*	*Stem*	*Leaves*	*Solution*	*Loss*
1	1	21.0 (5.3)	6.9 (2.5)	0.0 (0.0)	17.3 (6.4)	54.9 (8.6)
	5	12.0 (3.2)	2.9 (0.7)	0.1 (0.1)	41.2 (8.0)	43.7 (11.1)
	10	10.3 (2.9)	2.8 (1.7)	0.4 (0.1)	43.0 (13.9)	43.5 (14.4)
2	5	1.3 (0.3)	2.2 (1.0)	0.0 (0.0)	63.2 (12.8)	33.3 (9.8)
	10	2.4 (1.3)	1.7 (1.3)	5.9 (5.0)	80.1 (12.7)	9.9 (13.3)
3	5	5.2 (5.5)	7.5 (6.6)	0.8 (0.5)	36.1 (13.7)	50.4 (11.3)
	10	1.9 (0.5)	5.1 (3.2)	1.3 (0.5)	50.9 (12.0)	40.8 (4.6)
4	5	7.1 (5.5)	3.4 (1.4)	0.3 (0.3)	68.2 (6.3)	21.0 (8.3)
	10	3.1 (1.3)	4.0 (1.9)	0.6 (0.3)	75.4 (5.4)	16.9 (5.6)
5	5	21.2 (6.7)	3.5 (1.8)	0.2 (0.1)	56.2 (9.3)	18.9 (3.6)

4.3 Uptake and loss

If CN is taken up into the plant the question is whether it is metabolized or remains in the plant as an iron cyanide complex?

A mass balance for CN in the study by Larsen and Trapp (2005) showed that 20-83% of the CN was removed from the solutions while 10-55% of the CN was lost from the system (plant + solution) (Table 4.1). The highest percentage removal was found in the solutions with ferricyanide and with no additional source of nitrogen. The smallest removal was found in the solution with ferrocyanide. The percentage removal from solution was higher at low concentrations, similar to Michaelis-Menten uptake kinetics. The two facts together - increased uptake of cyanide at nitrogen deficiency and concentration-dependent uptake - implies an involvement of plant metabolism in the uptake process.

Due to the very low concentrations of free CN in both experiments and controls, the main part of the CN was considered lost due to metabolism inside the plant. The detection of CN in stem and leaves confirms that iron cyanide was taken up since it has recently been shown by Larsen et al. (2004) that free CN does not accumulate in healthy trees. The results are in agreement with results from Ebbs et al. (2003) where the use of ^{15}N labeled CN showed that 27% of the CN was lost from the solution while 8% of the CN was found in the plant tissue after 20 d when willow trees (*Salix eriocephala* L. var. Michaux) were grown in a ferrocyanide solution at 2 mg/L as CN.

Larsen and Trapp (2005) also measured total iron concentrations in solutions and plants and found that the final concentration of iron in trees exposed to iron cyanides was in all except one case significantly higher than in controls. Similar to total cyanide, the highest concentrations of total iron were found in roots. Since iron cyanide was the only external source of iron, an increase of iron in the plant material should be caused by uptake of iron cyanide complexes or iron from decomposed iron cyanide complexes. Given that the decomposition of the iron cyanide complexes was very slow and that very small concentrations of free CN were measured in both controls and experiments, the increase of total iron in plants exposed to iron cyanide suggests that the iron was taken up as iron cyanide complex. They also used the mass ratio of iron to cyanide inside the plant to confirm that the iron cyanide complex is decomposed inside the plants, and subsequently, the free cyanide is metabolized.

5 Discussion and conclusion

The speciation of CN at MGP sites is dominated by the two solid complexes Prussian blue and Turnbull's blue. The complexes are stable at the low pH found in CN polluted soil at MGP sites but the dissolution increases with increasing pH. By dissolution Prussian blue and Turnbull's blue releases the two water soluble iron CN complexes ferro- and ferricyanide. According to equilibrium calculations ferro- and ferricyanide complexes are only stable at pH > 7.

5.1 Risk assessment

Only at high pH levels in the soil are ferro- and ferricyanide complexes considered to constitute a risk. An increase of pH will increase leaching of the complexes to the groundwater and if the groundwater is exposed to sunlight before consumption, free CN can be formed.

The risk of ingestion of CN polluted soil is difficult to predict since it will change from site to site depending on concentration, geology, climate etc. Both low and high risk evaluations can be found in the literature but in general it must be concluded to be site specific.

Volatilization of free CN is only considered to constitute a risk in closed environments where the soil can be exposed to sunlight, e.g. greenhouses. Plant uptake of CN is not considered as an important exposure route for eatable crops growing above the soil surface while CN concentrations in root vegetables growing in the soil, like carrots and radish, should be further investigated.

5.2 Uptake, metabolism and toxicity of free cyanide in plants

All vascular plants have an enzymatic system to metabolize CN formed during ethylene synthesis. Metabolism of artificial supplied CN was investigated with a phytotoxicity test and a metabolism test. Five different woody plants (willow, poplar, birch, elder and rose) were all able to remove free CN (HCN and CN^-) from a solution.

The fastest removal was seen for willow trees (*Salix viminalis*) where removal rates for leaves and roots were around 9.5 and 7 mg/kg/h, respectively.

Willow trees grown in a hydroponic solution containing free CN started to show phytotoxic effects at concentrations of 2 mg/L. When grown in sand the phytotoxicity was approximate a factor 10 lower.

> 90% of the free CN supplied to the willow trees was removed from the system and no accumulation of CN was found in healthy plants.

A non-linear mathematical model was used to balance estimates of uptake and metabolism. The model predicted that at low doses (< 10 mg/L when grown in sand), the CN would be rapidly metabolized. At higher doses, uptake would be faster than metabolism and consequently CN would accumulate in the plant tissue.

5.3 Plant uptake of iron cyanide complexes

When grown in hydroponic solutions containing ferro- or ferricyanide at concentrations up to 10 mg/L as CN, willow trees showed no phytotoxic effects. The speciation in the solutions became dominated by ferrocyanide during the experimental period when an electron donor was present. It appears that the removal from the solution decreases as the concentration of ferrocyanide increases suggesting that uptake of ferricyanide is preferred over uptake of ferrocyanide. Increased concentrations of CN were found in all plant compartments and between 10 and 50% of the CN were lost from the system. The highest percentage removal was found in the solutions with ferricyanide and with no additional source of nitrogen which implies that lack of nitrogen increases the uptake and decomposition of iron CN.

5.4 Interpretation

A full scale phytoremediation project with willow trees growing in CN polluted soil has been implemented at the former Søllerød MGP. The outcome of the project has not yet been evaluated. The role of the willow trees is to decrease the leaching of contaminants to the groundwater by transpiration and to remove contaminants from the soil by plant uptake.

Results from this Ph.D. project have shown that plant uptake and metabolism of both free and complex CN is possible, thus the limiting factor for phytoremediation of CN polluted soil is probably the dissolution of the solid iron CN complexes. Since the solubility of Prussian blue increases with increasing pH, an increase of soil pH, eventually by spreading of lime, is very likely to increase dissolution and plant uptake of iron CN complexes. In addition, an increase of soil pH can be necessary for the plants to survive in the typically very acidic soil.

An increasing solubility of iron CN complexes also increases the possibility of leaching of CN to the groundwater; hence, the transpiration of the willow trees should be sufficient to prevent migration of water from the unsaturated zone to the groundwater

6 Outlook

The work included in this thesis provided a basis for continued investigations within the following topics.

- **Uptake of cyanide complexes in eatable crops.** Sorption or uptake of cyanide complexes in eatable crops, in particular root crops, might be an important exposure route for CN. Field data for crops growing in cyanide polluted soil are defective. A study with growth of different vegetables, e.g. carrots, radish, lettuce, in CN polluted soil for example soil from an MGP site, could give valuable information on the exposure route.

- **Uptake and leaching of CN from MGP soil**. A column experiment with willow trees growing in MGP soil could give useful information on the uptake and leaching of CN compounds. The pH in the soil and the amount of irrigation should be varied to determine to what extend the willow trees can prevent leaching.

- **Phytoremediation of mining wastewaters.** Large amounts of cyanide are used in the mining industry to extract precious metals from ore. As suggested by Trapp et al. (2003) cyanide can be removed from the mining wastewater by plants. A pilot plant to study the efficiency and the economy of this treatment method could open for a more environmentally compatible mining.

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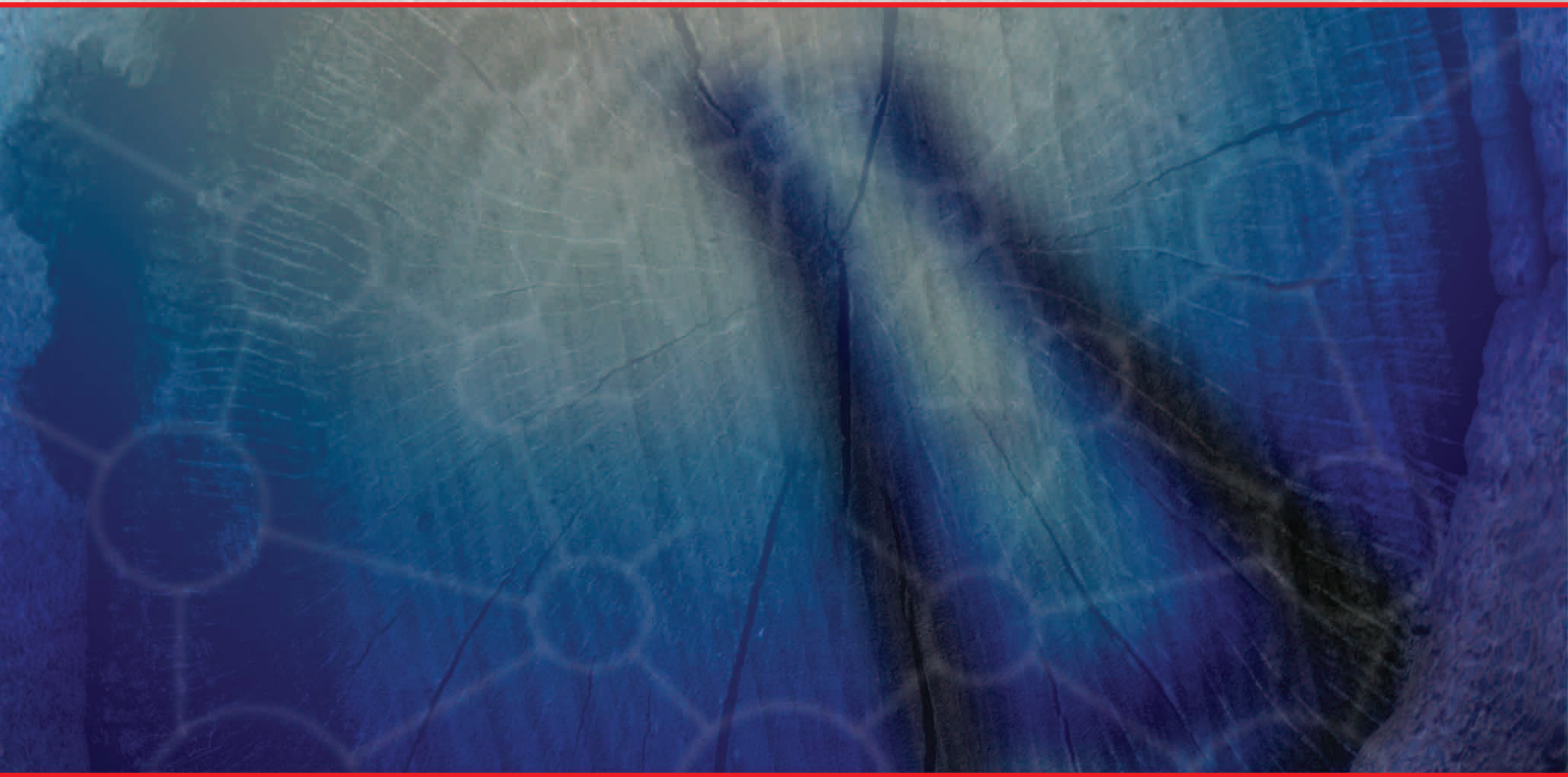

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