PHYTOREMEDIATION OF SOIL AND GROUNDWATER CONTAMINATED BY CHLOROBENZENE AND BENZENE USING *EUCALYPTUS UROGRANDIS* AND *PINUS TAEDA*

by

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(Under the Direction of Aaron Thompson and Lawrence A. Morris)

ABSTRACT

Soil and groundwater at a former pesticide factory in southern Brazil was contaminated by chlorobenzene and benzene, and we evaluated phytoremediation using trees. In a greenhouse experiment, we exposed *Eucalyptus urograndis*, *Pinus taeda*, and no-plant controls to an aqueous solution of chlorobenzene and benzene (50 mg L^{-1} each). Both trees enhanced contaminant mass removal from 5 to 50% relative to no-plant controls. In a field experiment in Brazil, the planted *Eucalyptus urograndis* reached 5 m height within one year in the presence of these contaminants. We estimate these trees could remove up to 4.53 and 0.93 kg $year^{-1}$ of chlorobenzene and benzene, respectively, in the field experiment. Our work suggests *Eucalyptus urograndis* and *Pinus taeda* are strong candidates for accelerating the remediation of chlorobenzene and benzene in soil and shallow groundwater.

INDEX WORDS: Phytoremediation, chlorobenzene, benzene, contaminant removal enhancement, Eucalyptus, Pinus, phytopumping, persulfate.

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DEDICATION

I would like to dedicate this thesis to my entire family (especially my father José Geraldo Barcellos and my mother Eliane Casotti Barcellos), my close friends, my girlfriend and her family for all their support, prayers, and life lessons.

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CHAPTER 1

OVERALL INTRODUCTION & LITERATURE REVIEW

1.1. Overall Introduction

Environmental contamination by chlorobenzene and benzene is a common problem worldwide. The availability of cost-effective technologies, such as phytoremediation, is growing and may help to reduce the use of more costly approaches to remediating soils/groundwater, such as soil excavation, washing/burning, and groundwater pump-and-treat approaches (Pilon-Smits, 2005). Phytoremediation projects can cost in general 50 to 90% less than traditional remediation technologies, such as physical, chemical, and thermal techniques (Rock and Sayre, 1998).

The soil and groundwater at a former pesticide manufacturing facility in a terraced floodplain in southern Brazil (state of Rio Grande do Sul – RS) was contaminated by several benzene-based compounds (chlorobenzene, benzene, etc.). The contamination was a consequence of storage tank leaks, formulation processes, and inappropriate disposal practices. Given the environmental and waste characteristics of the site, phytoremediation was proposed as a combined remediation strategy together with chemical oxidation to clean up the pollutants at this site.

The research presented in this thesis has the following objectives:

(1) Evaluate plant survival when exposed to high concentrations of chlorobenzene and benzene as well as when exposed to a chemical oxidant (sodium persulfate);

(2) Assess the capability of *Eucalyptus urograndis* and *Pinus taeda* to enhance removal of chlorobenzene and benzene in contaminated soils, under greenhouse conditions;

(3) Evaluate the phytoremediation potential and growth of *Eucalyptus urograndis* in a chlorobenzene/benzene contaminated site in southern Brazil;

(4) Assess the effects of *Eucalyptus urograndis* plants on the groundwater movement and water table level fluctuation at this site in Brazil.

This thesis contains four chapters and an appendix as follows:

- Chapter 1 – Overall Introduction & Literature Review;
- Chapter 2 – *Eucalyptus urograndis* and *Pinus taeda* Enhance Removal of Chlorobenzene and Benzene in Contaminated Soils: A Greenhouse Study;
- Chapter 3 – Potential for Phytoremediation by *Eucalyptus urograndis* at a Site Contaminated by Benzene-based Compounds in Southern Brazil;
- Chapter 4 – Overall conclusions.
- Appendix A – Contains the greenhouse survivorship study of *Eucalyptus urograndis* exposed to chemical oxidant (sodium persulfate).

1.2. Literature Review

1.2.1. Environmental Behavior and Health Risks of Chlorobenzene and Benzene

Organic pollutants are released into the environment by a variety of routes (spills, industrial manufacturing, wood treatment, poor disposal practices, etc.). The physicochemical and structural properties of the pollutant determine if plants can influence their removal from the soil. Most importantly, the pollutant's hydrophobicity

(K_{ow}) and volatility are key factors guiding selection of the best remediation designs (Nzengung, 2012). Benzene-based compounds (e.g., chlorobenzene and benzene) are used widely as precursors for the synthesis of pesticides and industrial chemicals and are common pollutants worldwide. Chlorobenzene and benzene have different behaviors in the environment and represents different risks to human health.

Chlorobenzene (CAS No. 108-90-7) is a colorless liquid with aromatic odor, high lipophilic characteristics, low water solubility, and with a log K_{ow} between 2.18 to 2.84. It is relatively mobile in the soil, and can be expected to leach into the groundwater, and/or to be adsorbed in soil organic matter and accumulated in the fatty tissue of organisms (Feidieker et al., 1995). When biodegraded, chlorobenzene forms the byproducts 2-chlorophenol and 4-chlorophenol (USEPA, 1995). Chlorinated-benzene compounds have a high potential to volatilize from soil pore space. Pure chlorobenzene, which has a vapor pressure of 12 mm Hg at 25^{o}C and a half-life of 21 days in the atmosphere, has a calculated half-life of 0.3 to 12 days in the soil (Howard, 1991).

Chlorobenzene is used as a chemical intermediate in the synthesis of pesticides, and as a solvent for adhesives, drugs, rubber, paints, and dry cleaning. Liver or kidney damage can occur for people that drink water containing chlorobenzene in concentrations more than the maximum contaminant level (MLC) for many years (USEPA, 2000).

Benzene (CAS No. 71-43-2) is a clear, volatile, and flammable liquid. The vapor pressure is 92 mm Hg (at 25^{o}C), and the log K_{ow} is 2.13. It is one of the BTEX components (benzene, toluene, ethylbenzene, and xylene), which are petroleum pollutants with similar fate in the environment (ATSDR, 2007). Benzene can be mobilized in the soil until reaches the water table, forming a light non-aqueous phase

liquid (LNAPL) plume. Benzene adsorbs to the soil, with most adsorption taking place in the soil organic matter. In addition, given its high vapor pressure, high air–water partition coefficient and moderate K_{oc}, benzene can volatilize from soil, especially in high porosity soils (International Program on Chemical Safety - IPCS, 1993). Benzene is also biodegraded in the environment under both aerobic and anaerobic conditions, but most effectively in aerobic environments. The European Chemicals Bureau (2007) estimated a half-life of 30 days for benzene in soil. Benzene can be absorbed by plant roots, particularly in sandy soils with low organic matter content (Wang and Jones, 1994).

Benzene is used as an intermediate for the synthesis of pesticides, and as a solvent for fats, waxes, resins, oils, inks, paints, plastics, and rubber. Benzene is known as human carcinogen for all routes of exposure. High levels of benzene inhalation can cause unconsciousness, drowsiness, dizziness, and headaches (USEPA, 2013).

1.2.2. Phytoremediation mechanisms for chlorobenzene and benzene contaminants

The use of plants and their associated microorganisms to achieve contaminant degradation, stabilization or attenuation is defined as phytoremediation (Arthur et al., 2005), which is an emerging, cost-effective alternative to traditional remediation methods of soil excavation, washing/burning, and groundwater pump-and-treat. Phytoremediation is considered a green remediation technology with a very small foot print (USEPA, 2008), with many advantages and disadvantages (Table 1.1).

Table 1.1. Summary of advantages and disadvantages of phytoremediation technology

Advantages	***Disadvantages***
Cost-effective	Plant growth is limited by the site environmental conditions
Aesthetic applicability and social acceptance	Effectiveness limited to shallow soil and groundwater
In situ remediation (low environmental impact)	It takes a relative long time to achieve site clean-up goals
Reduced final waste production	Variability on results
Multiple mechanism of remediation	Some mechanisms are not clearly understood

Yifru and Nzengung (2008) conceptualize five categories of phytoremediation (Figure 1.1): (1) rhizodegradation, where plant roots and associated microbial flora degrade pollutants; (2) phytoaccumulation, where the plant accumulates the contaminants in their tissues; (3) phytovolatilization, where plants mediate the volatilization of the contaminants; (4) phytodegradation, where plant enzymes achieve pollutant degradation; and (5) phytostabilization, where plants reduce the contaminant bioavailability through absorption and precipitation in soils.

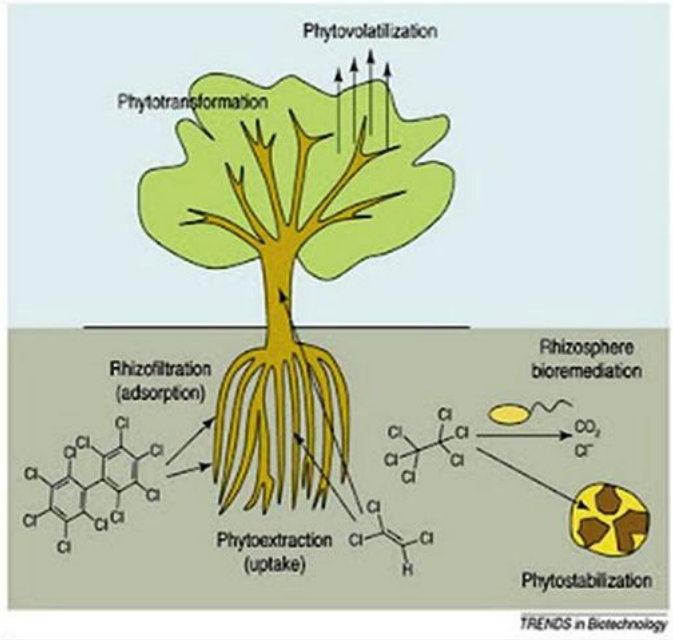

Figure 1.1. Illustration of Phytoremediation Mechanisms.

The main mechanisms of phytoremediation for chlorobenzene and benzene are: rhizodegradation, plant uptake, and phytovolatilization.

Rhizodegradation

Although plants do not directly uptake or degrade some pollutants, most plants have a mutualistic relation with microorganisms, which can contribute to the degradation of benzene-based compounds in the rhizosphere. Plants provide carbon to microbes through their exudates, which are able to degrade the pollutants in the rhizosphere. In addition, organic acids (e.g., citric or malic acids) exuded into the soil by plants can provide desorption and solubilization of pollutants, making the pollutants available to be taken up and/or degraded by plant roots and microbes (Nzengung, 2012). The acidification of the rhizosphere is caused by a combination of root release of protons and organic acids and microbial oxidation of organic compounds (Jones, 1998). Plant enzymes, such as nitroreductase and lactase, have shown their ability to break down aromatic compounds, while the dehalogenase has been able to reduce chlorinated molecules into chloride ions, carbon dioxide, and water (Schnoor et al., 1995).

Because rhizoremediation is mediated by both the plant roots and associated microorganisms, an important aspect is to select plants with a large root mass in order to obtain high rates of rhizodegradation and good consortia with soil microbes (Nzengung, 2012). The type of microbes in the rhizosphere depends on several factors such as: root exudates, plant species, soil characteristics, site history, and other environmental factors (Da Silva et al., 2006).

Petroleum hydrocarbons (including benzene) are some examples of organic compounds degraded by rhyzodegradation (Hutchinson et al., 2003). Kamath and co-

workers (2004) describe some potential rhizosphere interactions for petroleum hydrocarbon remediation: microbial growth, repression or induction of catabolic enzymes, co-oxidation of contaminants, and changes in bioavailability.

Adsorption may be significant for some hydrophobic or organophilic organic contaminants, including chlorobenzenes, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and polychlorinated dibenzodioxins (PCDD). These compounds may accumulate on root surfaces and remain there bound to lipids in cell walls (Duarte-Davidson and Jones, 1996). As a consequence, transference into the plant following retention on root surfaces will be very slow. Due to their high persistence, once these contaminants reach the root surface they may effectively be bound for the lifetime of the plant and only very slowly be transported to other parts of the plant (Duarte-Davidson and Jones, 1996).

Uptake & Translocation

Organic pollutants are usually xenobiotic to plants, so the plants tend not to have specific transporters in their membrane cells; as a result, organic pollutants move into plants by simple diffusion. Moderately hydrophobic compounds (log K_{ow} = 0.5 to 3.0), such as benzene, can be removed from contaminated soil by plants through uptake process, as these compounds are hydrophobic enough to move through the lipid bilayers and soluble enough to travel into the cell fluid (Kamath et al., 2004). In addition, very hydrophobic compounds (log K_{ow} > 3) would be adsorbed to the external membranes of plant cells.

The ratio between the pollutant concentration in the xylem and the concentration in the external solution determines the translocation of contaminants from roots to shoots

and is quantified as the transpiration stream concentration factor – TSCF (Pilon-Smits, 2005). For some organic pollutants, like benzene, translocation inside plants may also happen through diffusion. Benzene has a high translocation rate from roots to shoot, can enter the leaf from shoot xylem, and can be degraded, accumulated, or volatilized to the atmosphere. For the case of accumulation, the three phases include chemical modification, conjugation, and sequestration. Degradation of the compound is more complex and dependent on the enzymes involved in the process (Environment Agency, 2009). Although chlorobenzene is primarily remediated in the rhizosphere, some studies reported that this contaminant can be taken up by plants, metabolized or volatilized from leaves to the atmosphere (Ma and Havelka, 2009; Braeckevelt et al., 2008).

Phytovolatilization

Volatile compounds with Hi > 10^{-6} (Henry's law constant) can be mobilized from the soil, taken up by plants, and volatized to the atmosphere by the process of phytovolatilization (Bromilow and Chamberlain, 1995). Given its high transpiration rate, Poplar is the most-used species for remediation of VOCs (mainly benzene), through the process of phytovolatilization (Pilon-Smits, 2005).

1.2.3. Phytopumping

In addition to phytoremediation, plant systems can also play an additional role in remediation of contaminated site through their ability to absorb and transpire water in a process termed phytopumping (or hydraulic control). Therefore, plants can be used to alter hydraulic gradients and to form a hydraulic barrier that helps stabilize contaminant

plumes and/or maintain an upward gradient to forestall downward contaminant leaching (Figure 1.2) (Pilon-Smits, 2005).

According to Kamath et al. (2004) poplars and willows are able to form a successful hydraulic barrier for contamination plume in 3-4 years (canopy closure) when planted densely. Mature poplar trees (*Populus* spp.), for example, can transpire 200–1000 liters of water per day (Wullschleger et al., 1998). *Eucalyptus* sp. is a fast growing tree that has a potential to be used for phytompumping and phytoremediation purposes, although it has not been explored much for such purposes. Some studies have estimated the evapotranspiration of Eucalyptus species; for instance: Dye (1987) found a range of 2.40 to 8.60 mm day^{-1} for trees with 22.0 m of height, Facco (2004) found 2.90 to 3.40 mm day^{-1} for Eucalyptus with 2 to 4 years old, and Sacramento Neto (2001) calculated an evapotranspiration of 8.60 to 10.00 mm day^{-1} for the 2 years old trees. *Eucalyptus* sp. is in the family Myrtaceae, order Myrtales and class Magnoliopsida. The plant came originally from Australia, and has been breed on the last decades to improve productivity. In Australia, they are the dominant tree of the higher rainfall areas of the country, and sparsely represented in the driest regions. There are nearly 900 species which have adapted to nearly every environment.

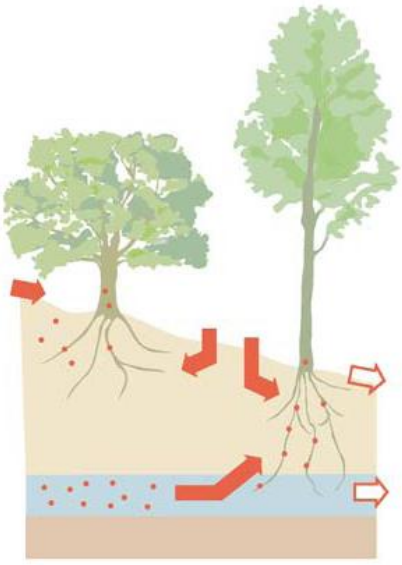

Figure 1.2. Phytopumping process (Pilon-Smits, 2005).

1.2.4. Examples of phytoremediation for chlorobenzene and benzene

Several examples of successful tree-based phytoremediation projects involving chlorobenzene and benzene exist. Willow trees (*Salix* spp.) planted in a petroleum-contaminated site reduced benzene by 90% of its initial concentration of 65 mg L^{-1} in groundwater and 2500 mg L^{-1} in soil (Nzengung, 2005). Using hydroponic experiments, Burken and Schnoor (1998) showed that poplar trees (*Populus deltoides*) extracted 94% of ^{14}C labeled benzene and ^{14}C-1,2,4-trichlorobenzene. Absorbed benzene was quickly translocated, and transpired from the leaves. There are also some other examples of projects involving phytoremediation of benzene in contaminated sites (McLinn et al., 2001; Green and Hoffnagle, 2004).

Successful phytoremediation has also been documented in wetland systems. Braeckevelt et al. (2011) found that common rush (*Juncus effusus*) in planted fixed-bed reactors decreased chlorobenzene by 99% of its initial 16.6 mg L^{-1} concentration. Other constructed wetland systems have decreased chlorobenzene (CB) and 1,4-

dichlorobenzene (1,4-DCB) by 48% (initial concentration 25 mg L^{-1}) and 38% (initial concentration 0.25 mg L^{-1}), respectively (Braeckevelt et al., 2008). Removal was even greater in the surface layer of the wetland, with 71% removal of CB and 62% removal of 1,4-DCB; however, the co-contaminant 1,2-dichlorobenzene (1,2-DCB), present at a concentration of 0.19 mg L^{-1}, was not removed (Braeckevelt et al., 2008). In a phytoremediation experiment with planted vertical biofilters, the use of Reed (*Phragmites sp*) was responsible for a total decrease in chlorobenzene contamination from 12,700 mg L^{-1} to 0.13 mg L^{-1} (Denecker, 2009).

Other phytoremediation projects have shown that chlorobenzene is removed from contaminated soil and groundwater mainly by rhyzodegradation and phytovolatilization (Imfeld et al., 2009; Gomez-Hermosillo et al., 2006). The lipophilicity and volatility of chlorobenzene makes it a good candidate for phytoremediation. It can be absorbed by plant roots, particularly in sandy soils with low organic matter content (Wang and Jones, 1994).

There is not a specific study showing the use of *Eucalyptus* sp. and *Pinus* sp. to remediate soils and groundwater contaminated by chlorobenzene and/or benzene. However, regarding the use of loblolly pine (*Pinus taeda*) for phytoremediation of VOCs, a study in the Savannah River Site (SC, USA) shows removal of trichloroethene and cis-1,2-dichloroethene in shallow groundwater (Vroblesky et al., 1999). Results showed that the presence of trees provided a reduction of trichloroethene of 30-70% of its initial concentration (around 3000 nmol L^{-1}), and the trees also accumulated both cis-1,2-dichloroethene and trichloroethene in their tissues. On the other hand, *Eucalyptus cineria* were used to decontaminate perchlorate-contaminated water, but it had low survivability in the hydroponic system, despite that willow trees were the most favorable plants to

remediate perchlorate in this study (Nzengung et al., 1999). However, no study tested the phytoremediation potential of *Eucalyptus grandis*, *E. urophylla*, and their hybrid *E. urograndis*.

Therefore, the examples and the processes explained above illustrate that phytoremediation can be a feasible option for remediation of contaminated sites by organic compounds including chlorobenzene and benzene.

CHAPTER 2

EUCALYPTUS UROGRANDIS AND *PINUS TAEDA* ENHANCE REMOVAL OF CHLOROBENZENE AND BENZENE IN CONTAMINATED SOILS: A GREENHOUSE STUDY[1]

[1] Barcellos, D., L.A. Morris, T. Moura, V. Nzengung, N. Mantripragada, and A. Thompson. To be submitted to Journal of Environmental Quality.

Abstract

Contamination of soils and groundwater by chlorobenzene and benzene is a common problem at industrial sites throughout the world. Chemical remediation techniques are rarely completely effective, and thus remnants of these contaminants often persist at levels that can still impact ecosystem function. In this study we evaluate the potential of two tree species to accelerate the removal of these compounds from soil/water systems, as well as for the trees themselves to survive exposure to the contaminants. To first evaluate tree survival, we exposed *Pinus taeda* seedlings to a range of chlorobenzene concentrations up to 1.0 g L^{-1} and benzene concentrations as high as 2.0 g L^{-1} (in water), both well above the compound's aqueous solubility limit. We did not observe mortality in any of the treatments, but rather found that all plants grew similarly by gaining 2.0±0.8 cm of height over a 30-day experiment. We then conducted a completely randomized block greenhouse experiment using *Pinus taeda, Eucalyptus urograndis,* and a non-planted control in custom-built pots designed to mimic groundwater rise in a sand media. At two-day intervals, we added a contaminant solution containing 50 mg L^{-1} of both benzene and chlorobenzene to each pot and sampled the leachate solution daily from the bottom of the pots through a valve. Aqueous contaminant concentrations were quantified by gas chromatography – mass spectrometry (GC-MS) following extraction into hexane. Both tree species enhanced mass removal of chlorobenzene and benzene in water from 5 to 50% of the initial contaminant mass added to each pot. We did not detect any contaminant in the plant roots/leaves or on the sand-substrate. Our results suggest these trees show promise for remediating chlorobenzene and benzene in soils and groundwater.

2.1. Introduction

Benzene-based compounds (e.g., chlorobenzene and benzene) are used widely as precursors for synthesis of pesticides and industrial chemicals, and are common pollutants worldwide (Ellis and Rivett, 2007). These compounds can be commonly found in groundwater due to industrial or petroleum spills (Schmidt et al., 2004). Chlorobenzene and benzene present a problem leading to soil contamination due to inappropriate disposal practices and accidental leakage from storage tanks or pipes within industrial production facilities. These contaminants may reach bodies of water and may cause multiple risks to humans and animals health, causing toxicity (mainly in the central nervous system), dizziness, headaches, etc., when high levels are inhaled in drinkable water or are encountered in the food chain (USEPA, 2000; USEPA, 2013). Chlorobenzene, for instance, has a low water solubility and high lipophilic characteristics that promote its adsorption to soil organic matter and accumulation in the fatty tissue of organisms (Feidieker et al., 1995). However, these compounds are also volatile and most efforts to remediate them focus on this property.

Conventional remediation technologies (soil excavation, washing/burning, and groundwater pump-and-treat approaches) are commonly used to remediate groundwater and soils at contaminated sites, but these techniques can be expensive and difficult to implement (Seeger et al., 2011). Thus, phytoremediation, which is the use of plants and their associated microorganisms to achieve contaminant degradation and/or attenuation (Arthur et al., 2005), is an emerging cost-effective alternative to these conventional remediation methods (Pilon-Smits, 2005), and can be a polishing treatment for residual contamination from other remediation techniques (e.g., in-situ chemical oxidation and/or

reduction). Phytoremediation projects can cost 25 to 40% of the total cost of conventional remediation techniques (Green and Hoffnagle, 2004).

Phytoremediation of contaminated sites can be achieved via one or multiple of the following mechanisms: rhizodegradation, phytoaccumulation, phytovolatilization, phytodegradation, and phytostabilization (Yifru and Nzengung, 2008). Many case studies of successful application of phytoremediation of VOCs are reported in the current peer reviewed literature (Burken and Schnoor, 1998; Ma and Havelka, 2009; Braeckevelt et al., 2008). Benzene and chlorinated benzene contaminated sites are more amenable to pytoremediation by primarily rhizodegradation, uptake and phytovolatilization, and uptake and phytodegradation.

A key factor in phytoremediation success is selecting and cultivating suitable plants that can survive when exposed to the pollutants in contaminated sites (ITRC, 2009). Some of the desirable plant characteristics for phytoremediation of volatile organic compounds (VOCs) are high evapotranspiration (ET) rate, ease of planting and maintaining in the field, and capability to transform contaminants into less toxic products (Schnoor, 1997). The trees *Eucalyptus urograndis* and *Pinus taeda* are potential candidates for long-term ecosystem restoration and for phytoremediation of VOCs, as they grow fast, have high ET rates, and adapt well to different environments. However, their potential to phytoremediate either chlorobenzene or benzene has not been demostrated.

This research is structured around two questions: (1) Can trees survive when exposed to contaminant concentrations typically found in polluted soils? (2) If they do survive, do they enhance removal of chlorobenzene and benzene in contaminated soils?

We addressed the first question by exposing *Pinus taeda* seedlings to a range of contaminant concentrations for 30 days. We addressed the second question by repeated exposure of *Eucalyptus urograndis* and *Pinus taeda* trees and no-plant controls to both chlorobenzene and benzene, and by monitoring the loss of those contaminants in a randomized block designed greenhouse experiment.

2.2. Materials and Methods

2.2.1. Experiment 1: Assessing *Pinus taeda* Seedlings Survival Exposed to Chlorobenzene and Benzene

To ensure the plants would survive in a 4-month greenhouse experiment (our second question), we first conducted a short-term study in a fume hood for 30 days aimed to test the survivorship of Pine (*Pinus taeda*) seedlings exposed to a range of contaminant concentrations. We selected Pine seedlings for this quick survivorship study because they were short enough to fit inside a standard fume hood, whereas the *Eucalyptus* sp. seedlings were too tall. The chosen contaminant concentrations were based on the concentration found in groundwater at a contaminated site in southern Brazil: 50 mg L^{-1} for benzene (BZ) and 100 mg L^{-1} for chlorobenzene (CB), which is the Treatment 1xT (Table 2.1). The solubility of these compounds in water (at 20^{o}C) is 1,800 mg L^{-1} for BZ and 500 mg L^{-1} for CB; thus, the concentrations at the treatment 1xT are within the solubility range for each compound. However, in the 40xT treatment, both chlorobenzene and benzene were not completely dissolved and we added them to the reactor as both dissolved phase and NAPL (non-aqueous phase).

The Pine seedlings were potted in small cones of PVC (20 cm high by 4.5 cm in diameter), containing an unfertilized commercial soil mix (Fafard 3M) and placed in a fume hood with two rows of fluorescent light (Figure 2.1). The plants were placed in a randomized block design with four-replicates of five treatments: 0 (control), 0.1xT, 1xT, 5xT, and 40xT. They were irrigated daily for 30 days with 50 mL of the four contaminant solutions and 50 mL of the control (tap water) treatment (Table 2.1). Seedling height was measured weekly, and possible signs of mortality were monitored daily.

Table 2.1. Treatments and concentrations used to test survivorship of *Pinus taeda*

Compound	**Concentrations (mg L^{-1}) in different reactors**					**Solubility in water at 20°C (mg L^{-1})**	**Density (g mL^{-1})**
	Control	*0.1xT*	*1xT*	*5xT*	*40xT*		
Chlorobenzene	0	10	100	500	1,000†	500	1.11
Benzene	0	5	50	250	2,000	1,800	0.875

† Concentration obtained by 10 x (1xT) instead of 40 x (1xT).

Fig. 2.1. General view of the survivorship experiment under the hood.

2.2.2. Experiment 2: Phytoremediation under greenhouse conditions

Experimental Design

We chose to use *Eucalyptus urograndis* and *Pinus taeda* as they are widely planted in southern Brazil, and also because of their potential for phytoremediation in different climatic regions: Eucalyptus can be used for phytoremediation in tropical and subtropical regions, while Pine can be used in subtropical and temperate regions. The *Eucalyptus urograndis* seedlings were provided by Arborgen Inc. (Summerville, South Carolina), while the *Pinus taeda* seedlings were provided by Georgia Pacific Inc. (Albany, Georgia).

The phytoremediation experiment was conducted in a greenhouse at the University of Georgia (Whitehall Forestry, Athens, GA, USA). The greenhouse temperature was maintained at 25 ± 3^{o}C and the relative humidity was 70 ± 5%. The trees were placed in a randomized block design replicated in four complete blocks, in order to distribute the treatments and replications over the greenhouse micro environmental conditions (Hammer and Douglas, 1997). Treatments were a factorial combination of three plant conditions (*Eucalyptus*, *Pinus* and no plant) and two contaminant conditions (contaminant solution or water) replicated in four complete blocks; thus, there were 6 treatments (3 with contaminated solution and 3 no-dosed controls) for a total of 24 pots (n=4 reps). The dosing solution was an aqueous mix containing both chlorobenzene and benzene at 50 mg L^{-1}.

The seedlings were planted in columns containing washed sand. Each column was made of cut PVC pipe capped at the bottom, linked by an outlet valve and a water head control that was made of clear PVC pipe (Figures 2.2 and 2.3). The 40-cm-tall pots (15

cm I.D.) were attached to a clear PVC tube (1.25 cm I.D.) placed vertically to control the height of water in the soil column. Washed sand was used to reduce contaminant sorption.

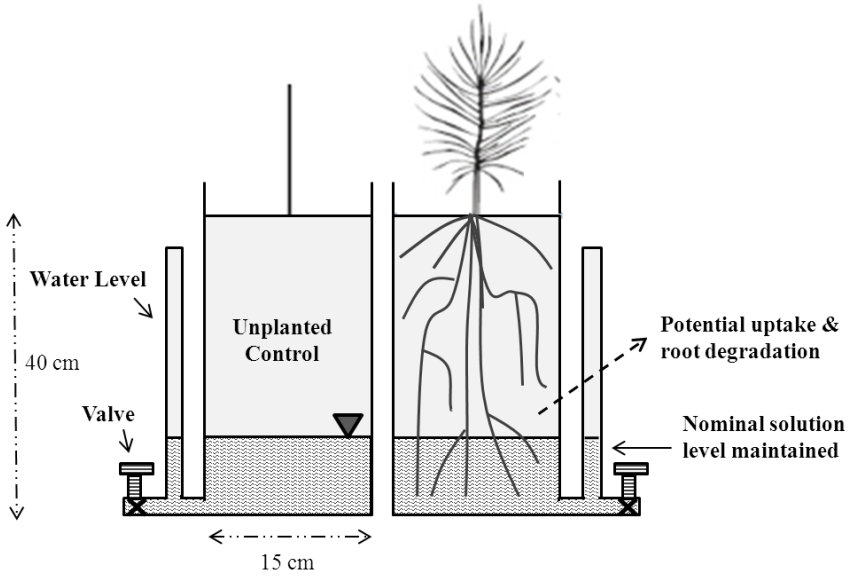

Fig. 2.2. Schematic of unplanted (Control) and planted pots in the greenhouse.

Fig. 2.3. Pots with Eucalyptus, Pine, and No-Plant in the greenhouse.

Trees were fertilized with Hoagland complete medium solution (Plant Media Inc.), at an application rate of 100 mL per week of a solution prepared as 1.6 g of Hoagland's solid media per L of water. The Hoagland's solution supplied N, P, K, Ca, Mg, S, B, Fe, Mn, Zn, Cu, and Mo to plants. This solution was applied in the no-plant pots as well.

Solution Application & Sample Collection

To simulate conditions where plant roots are in contact with both contaminants in a field site, we supplied the chlorobenzene/benzene aqueous solution at the bottom of the pots through the water head control pipe (stand pipe) for the treatments Eucalytpus, Pine, and non-plant, filling around 1/3 of pot height (Figure 2.2). As a result, the contaminant solution moved up into the root zone through a combination of capillary rise and transpiration.

Dosing in all greenhouse pots was initiated once the trees presented healthy growth (4 weeks after planting). The phytoremediation experiment lasted 4 months (August to December 2012). During each dosing event, a fresh solution was prepared in deionized water containing both chlorobenzene and benzene, each at a concentration of 50 mg L^{-1}. Based on preliminary dosing experiments, which were 7 uniform applications of contaminants in all pots, we established the following application/sampling strategy.

We applied the contaminant solution through the stand pipe, until the solution reached a constant level of 12 cm above the bottom of the pot and, immediately after, we collected an aqueous sample through the bottom valve. Then, we collected another sample within 24 and 48 hours after the first collection. However, between each

collection (24-hour period), we maintained the water level by adding fresh tap water every 3-8 hours to account for evapotranspiration in the planted pots, making sure that we sampled the solution 2-3 hours after the last water leveling. We did not add fresh water to the no-plant pots. Then, on day 2 (48 h), we withdrew all the contaminant solution in each pot by opening the valve. One hour later, we repeated this dosing/sampling procedure similarly for a second cycle, then for a third and fourth cycle. Nevertheless, the fourth cycle lasted longer: sample collection at 0, 24, 48, 72, 96, and 120 hours. Thus, the protocol sequence was applying contaminant solution on the first day of three sequential two-day cycles and on the first day of one final five-day cycle, with aqueous samples collected daily throughout the experiment. Each sample was collected in a Qorpak® glass bottle and quickly stored in a freezer.

In addition, we plugged a stopper (covered by aluminum foil) at the top of each stand pipe (water level pipe) to reduce contact between the contaminant solution and atmosphere. Evapotranspiration rates were recorded from changes in the water level observed in the clear external PVC pipe (measured with a ruler), which was hydraulically connected to the main pot (Figure 2.2).

Analysis of contaminated water

All aqueous samples were thawed and a 5 mL aliquot was subjected to a liquid-liquid extraction with 5 mL of hexane and 1 mL of saturated NaCl, and then shaken for 5 min in a 25mL separatory funnel (Wennersten, 2004; Pratt and Stevens, 1992). The NaCl was added to break any emulsion and concentrate the analytes in the organic phase through the salting out effect (Eganhouse and Calder, 1976). After shaking, the hexane

phase was collected in a separate 30 mL glass bottle, and the aqueous layer was re-extracted with an additional 5 mL of hexane (no additional NaCl) for 5 min. Both hexane extracts were pooled together for analysis. This procedure yielded an extraction recovery between 78% and 89%.

The hexane extracts were loaded in an AS 2000 autosampler from where 1.0 uL was withdrawn from each sample and injected into a Thermo Finnigan Trace Gas chromatograph (GC) connected to a Polaris Q Ion trap Mass Spectrometer (MS). A Restek Rtx-VRX column (60m x 0.32mm x 1.4 um thickness), recommended inEPA method 8260-B for VOC analysis (USEPA, 1996), was used to separate chlorobenzene and benzene prior to quantifying the analytes on the mass spectrometer (MS). The GC conditions were as follows: Inlet temp of 180^{o}C; oven gradient program: initial 40^{o}C (1 min), 10^{o}C min^{-1} to 220^{o}C (0.5 min), then 20^{o}C min^{-1} to 240^{o}C. The MS interface temp was maintained at 240^{o}C. The carrier gas (Helium) was set for a constant flow of 1.0 mL min^{-1}. In the chromatograms, the Retention Time (RT) was 11.5 minutes for benzene and 15.5 minutes for chlorobenzene.

Chlorobenzene was analyzed by running the instrument in single ion monitoring (SIM) mode (USEPA, 1996) while benzene was analyzed in ion trap MS^2 mode, which approximates analysis via tandem mass spectrometer (Busch et al., 1990). Samples were quantified from a standard curve obtained by injecting standards of chlorobenzene and benzene dissolved in hexane from 0.1 to 100 mg L^{-1}. Fresh standards were prepared twice a week. The compound concentrations were quantified by using Polaris Xcalibur software (ver. 1.3). For each sample, the mass of chlorobenzene or benzene was

calculated by multiplying the compound concentration (obtained from the GC-MS) by the volume of solution in the planted or unplanted pot.

Analysis of contaminated sand, plant roots and foliage

At the end of the greenhouse experiment, sand and root samples were collected from the bottom (0 to 10cm) and top (20 to 30 cm) parts of the pots from each of the three treatments exposed to contaminants, along with leaf tissue of the Eucalyptus and Pine trees. All materials were placed in plastic bags and quickly frozen. These samples were then analyzed as described below for chlorobenzene and benzene.

After defrosting, a 20g-root sample was placed in a 60 mL Qorpak glass bottle with 30 mL of hexane. Similarly, 10 g of contaminated sand was added to 30 mL of hexane in a 60 mL Qorpak glass bottle for extraction. Both roots and sand samples were extracted into hexane using ultrasonic extraction (in a sornicator) for 30 min (Ozcan et al., 2009; Dunnivant and Elzerman, 1988), and then stored in a 2 mL vial until analysis.

Leaf samples were thawed, ground and extracted in a sornicator, adapting the ultrasonic extraction methodology of Dunnivant and Elzerman (1988) and Ozcan et al. (2009). For each leaf extraction, 2 g of leaf tissue was added to 25 mL of hexane and placed in a 60 mL Qorpak glass for ultrasonic extraction. After this step, the leaf sample extracts were applied to a Florisil® cartridge, according to USEPA Method 3620C (USEPA, 2007), and an aliquot of the hexane extract was stored in a 2 mL vial until analysis. The recovery of the clean-up step with Florisil was 93%.

After the extractions for sand, root, and leaf samples, each individual hexane extract was analyzed following the same GC-MS settings previously described. Results

were expressed in ug of the compound per g of sample (sand, root, or foliage) in wet weight.

Seedling Growth

For the greenhouse experiment, seedling height (H) from the soil surface to the apical bud and diameter (D) at the root collar was measured bi-weekly during the experiment and growth expressed as the change in stem volume index (SVI) calculated by the change in diameter squared times height (D^2H).

2.2.3. Statistical Analyses

The statistical analysis of contaminant removal was done by repeated measures within days following each dosing event. For the survival and greenhouse experiments, the relative growth height among the different treatments was compared. The statistical analysis was made using ANOVA in SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

2.3. Results

2.3.1. Experiment 1: Assessing *Pinus taeda* Seedlings Survival Exposed to Chlorobenzene and Benzene

None of the seedlings exhibited any signs of stress or mortality (Figure 2.4) after 30 days of daily contaminant irrigation with concentrations exceeding the aqueous solubility at 1 g L^{-1} of CB and 2 g L^{-1} of BZ (Table 2.1). The plants grew an average of 2.0±0.8 cm during the 30-d experiment, with no significant difference in growth among

the treatments (p=0.92). In addition, root growth was observed visually in all treatments, but an increase in mass or length was not quantified.

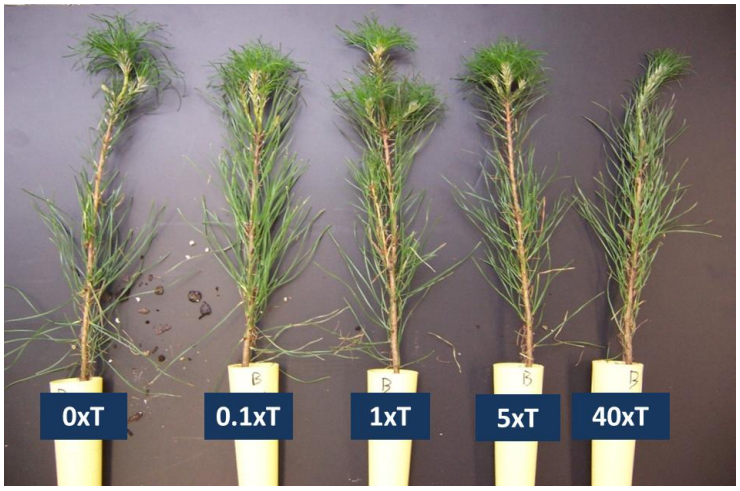

Fig. 2.4. Pine seedlings after exposure to chlorobenzene and benzene for 30 days. Control plants not exposed to contaminant (far left) and plants exposed to highest concentration (far right) did not show any mortality.

Results from this short-term experiment show that the chlorobenzene and benzene concentration used in the greenhouse study would not cause significant toxicity to seedlings. Although Pine was used in the latter study, we assumed that *Eucalyptus* sp. would also survive when exposed to the contaminants at similar concentration. With these results we proceeded to evaluate compound removal by plants (phytoremediation effect) during a four-month exposure of *Eucalyptus urograndis* and *Pinus taeda* to chlorobenzene and benzene, under greenhouse conditions.

2.3.2. Experiment 2: Phytoremediation under greenhouse conditions

As presented in Figures 2.5 and 2.6, the total mass (concentration x volume in the pot) of chlorobenzene and benzene declined over time for all treatments. However, the

rate of contaminant mass removal was greater in the presence of Eucalyptus or Pine (7 to 10 mg day^{-1}) than in the no plant control (3 to 6 mg day^{-1}).

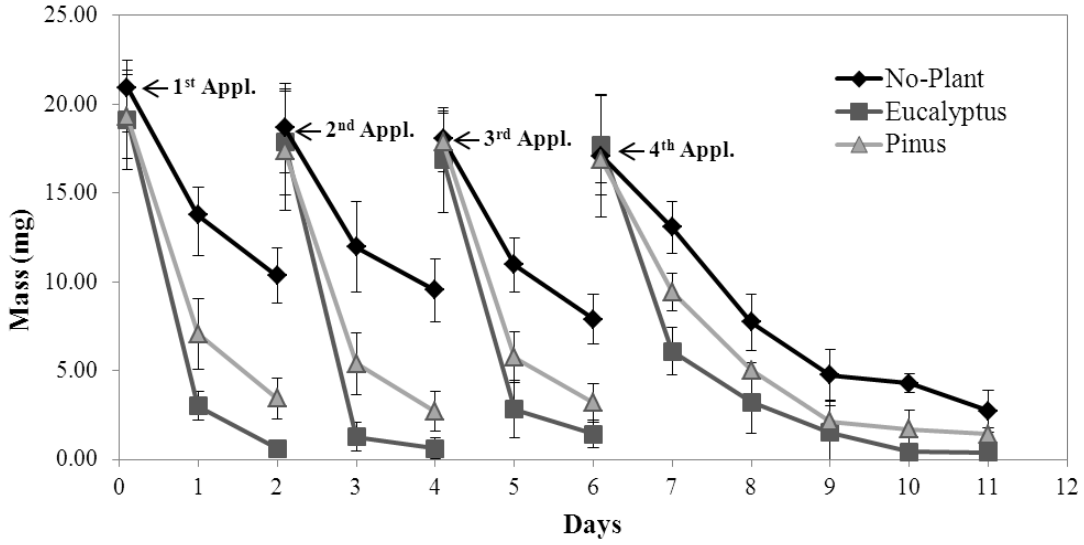

Fig. 2.5. Influence of Eucalyptus and Pine, versus a no-plant control during four applications (Appl.) of 50 mg L^{-1} of chlorobenzene in the greenhouse experiment. Error bars are ± 1 S.D. for n=4 reps per treatment.

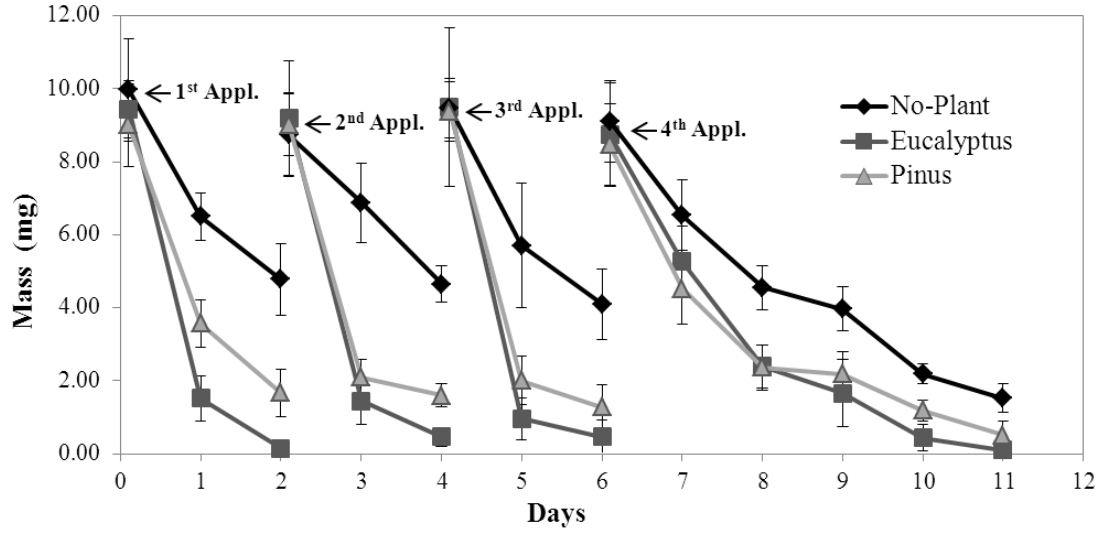

Fig. 2.6. Influence of Eucalyptus and Pine, versus a no-plant control during four applications (Appl.) of 50 mg L^{-1} of benzene in the greenhouse experiment. Error bars are ± 1 S.D. for n=4 reps per treatment.

Across all applications, the removal rates (in mg day^{-1}) were similar for chlorobenzene and benzene during the first two days following dosing. However, 5 days after dosing in the fourth application, the losses of both compounds in the no-plant treatment approached that of the Eucalyptus and Pine treatments (Figures 2.5 and 2.6).

To estimate the extent of CB and BZ removal due to the presence of the plants (Table 2.2), the pollutant concentrations of the non-plant treatment were subtracted from the Eucalyptus or Pine treatments on the second day of measurement for the first three applications and on day two and five for the fourth application. Thus, according to Table 2.2, the presence of trees indicates a phytoremediation effect accounting for removal of 1.3 to 10 mg of CB and 1.0 to 4.6 mg of BZ over 2 days, i.e. 5 to 50% of the initial contaminant mass was removed by plants. But, if we sum the plant remediation effect with the other losses of these compounds (potentially microbial remediation, volatilization, and sorption), we can observe 95-98% of total mass removal, for CB and BZ, in the soil columns in this greenhouse experiment (Figures 2.5 and 2.6). Therefore, *Eucalyptus urograndis* and *Pinus taeda* are capable of enhancing the remediation of contaminated soil/water in the environment.

A chromatogram for chlorobenzene and benzene for a random hexane extract is presented in Figure 2.7. In addition, no benzene or chlorobenzene was detected on the sand or plant roots and leaves.

Table 2.2. Contribution of Eucalyptus and Pine in enhancing mass removal of chlorobenzene and benzene (in the second day for the four applications, and in the fifth day for the fourth application).

		1st Appl. (2nd Day)	**2nd Appl. (2nd Day)**	**3rd Appl. (2nd Day)**	**4th Appl. (2nd Day)**	**4th Appl. (5th Day)**
Compound	**Plant**	Phytoremediation Effect† (mg)				
Chlorobenz.	Eucalyptus	9.7 ± 1.5***	8.9 ± 1.7***	6.5 ± 1.9***	4.5 ± 2.2**	2.3 ± 0.5*
	Pine	6.9 ± 2.7***	6.8 ± 2.9***	4.7 ± 2.5***	2.7 ± 2.0*	1.3 ± 1.5§
Benzene	Eucalyptus	4.6 ± 1.1***	4.2 ± 0.8***	3.6 ± 1.4***	2.2 ± 1.3*	1.4 ± 0.5*
	Pine	3.1 ± 1.6***	3.0 ± 0.8***	2.8 ± 1.6**	2.2 ± 1.3*	1.0 ± 0.8*

† Phytoremediation Effect = Mass of pollutant (no-plant treatment) - Mass of pollutant (plant treatment)
* Significant at the 0.05 probability level
** Significant at the 0.01 probability level
*** Significant at the 0.001 probability level
§ Not significant

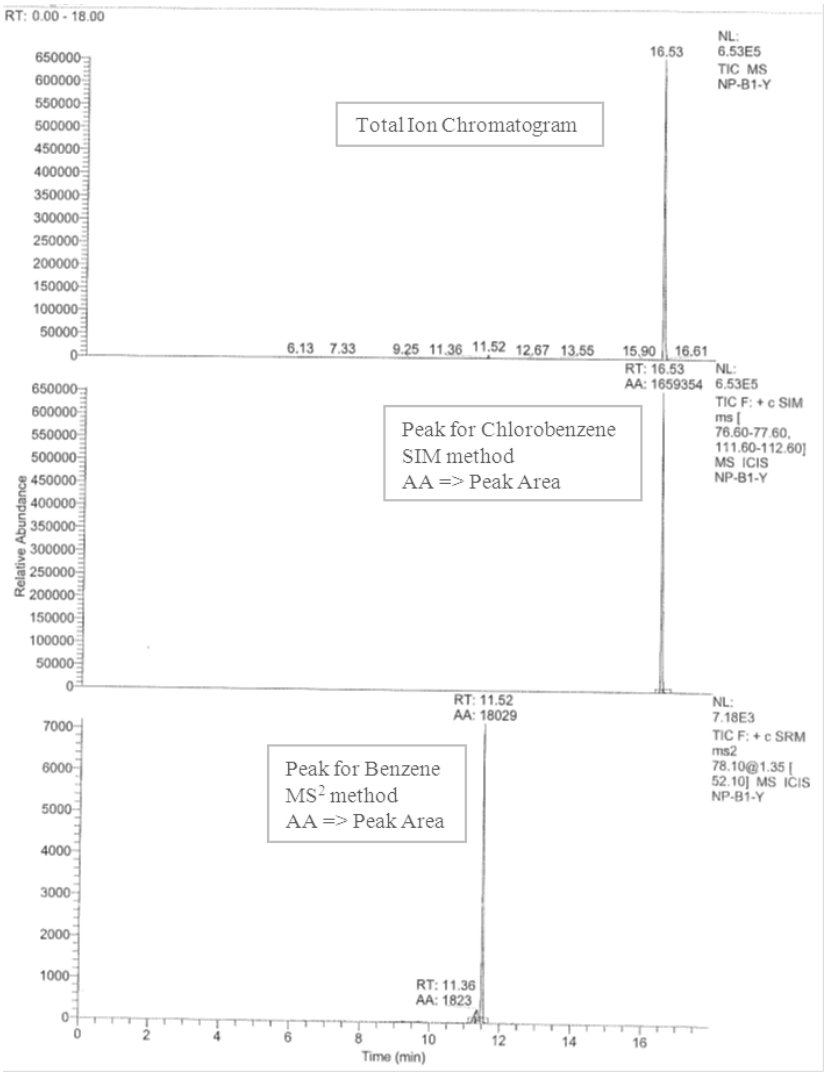

Fig. 2.7. Chromatogram for chlorobenzene and benzene extracted from solutions using hexane, showing the peaks and the areas (AA) of the analytes.

2.3.3. Growth response & Evapotranspiration rates

At the end of the greenhouse experiment, there was no significant difference in the height (p-value > 0.45), diameter (p-value > 0.48), or stem volume index (p-value > 0.37) of either eucalyptus or pine trees growing with or without chlorobenzene and benzene application (Figure 2.8). Similarly, the evapotranspiration rates for the treatments with and without both compounds were near constant (p-value > 0.35): 455 ± 72 mL day^{-1} for *Eucalyptus urugrandis* and 278 ± 59 mL day^{-1} for *Pinus taeda*.

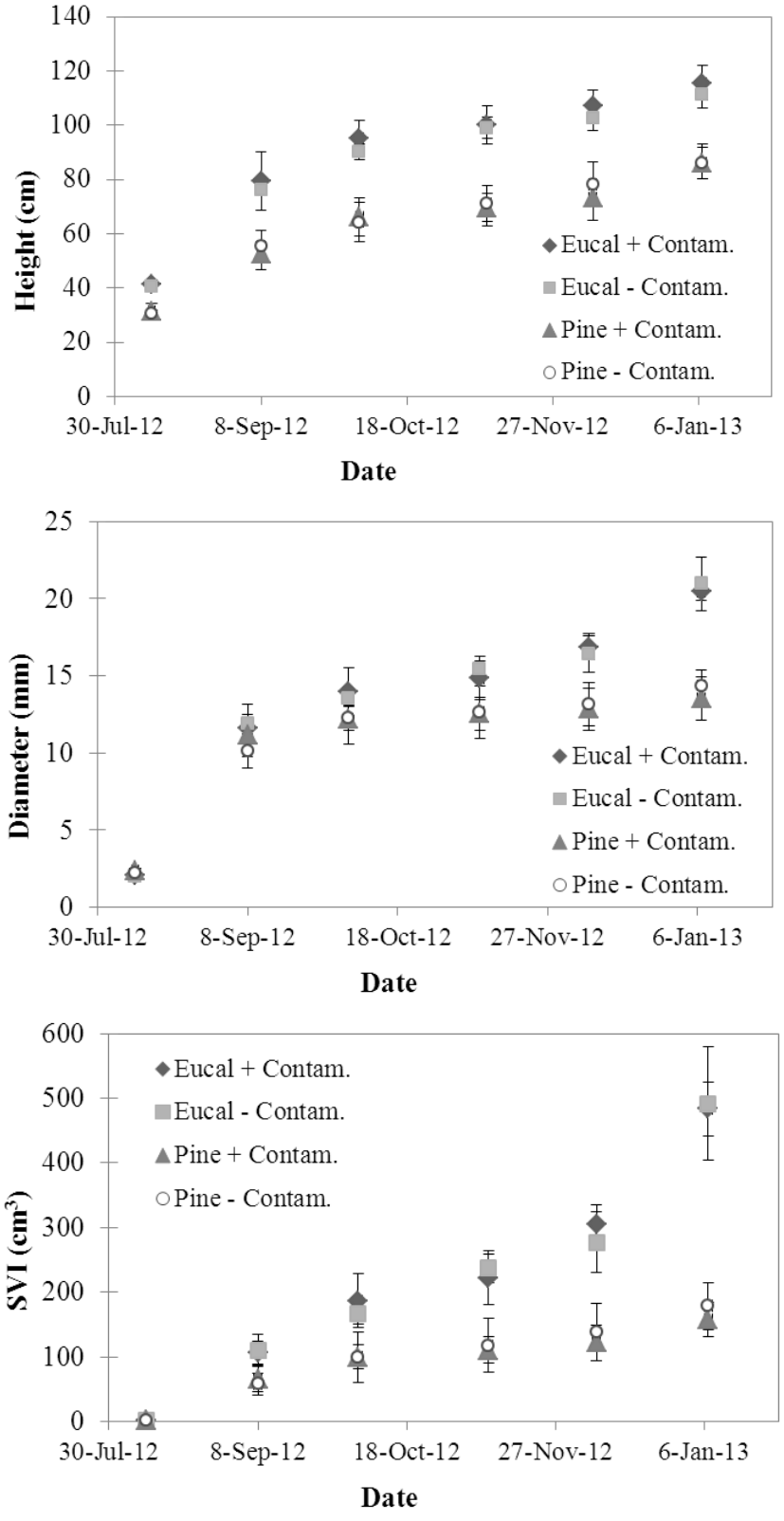

Fig. 2.8. Height, diameter, and stem volume index (SVI) for eucalyptus and pine exposed to chlorobenzene and benzene, or in absence of contaminants, during 5 months (August 2012 to January 2013), for four replications (n=4).

No visual or physiological differences among the treatments (contaminants and absence of contaminants) were observed (Figures 2.9 and 2.10), suggesting that plants can survive and grow in contact with these contaminants.

Fig. 2.9. Eucalyptus (left) and Pine (right) after five months in contact with a solution of both chlorobenzene and benzene.

Fig. 2.10. Eucalyptus (right) and Pine (left) root system after five months of exposure to both chlorobenzene and benzene.

2.4. Discussion

We used mass instead of concentration to show the contaminant removal (Figures 2.6 and 2.7) because we were counting the amount of mass added at the beginning and the amount lost at the end of each dosing cycle, taking in consideration the dilution factor, as we added water to the planted treatments because of transpiration. For both chlorobenzene and benzene, there was a higher amount (in mg) of contaminant removed by Eucalyptus and Pine in comparison to the no-plant control (Figures 2.5 and 2.6). Also, for both contaminants, it appears that plant uptake and volatilization were predominant in the first three cycles, and it appears that rhizodegradation was predominant in the fourth (last) cycle, as the difference between no-plant and plant treatments shortened (for this fourth cycle). This observation was made mainly because of the adaptation of microbes in the sequence of contaminant applications and, as a result, rhizodegradation was likely more intense in the fourth cycle.

Other studies have demonstrated that chlorobenzene can be remediated by the plants due to rhyzodegradation, microbial degradation, and also by phytovolatilization (Imfeld et al., 2009; Gomez-Hermosillo et al., 2006). Braeckevelt et al. (2011) found that common rush (*Juncus effusus*) in planted fixed-bed reactors decreased chlorobenzene by 99% of its initial 16.6 mg L^{-1} concentration, mainly by microbial degradation and phytovolatilization. On the other hand, benzene is remediated by plant uptake, phytovolatilization, phytodegradation, and some microbial degradation in the rhizosphere (Nzengung, 2005; Burken and Schnoor, 1998). According to Seeger et al. (2011), in a planted gravel filter/plant root mat experiment, benzene decreased 81 to 99% of its initial

concentration, although the benzene-removal was not fractionated between microbial degradation and plant remediation, as we are showing in our study.

Moreover, for the pot with no-plant, the contaminant removal rate (in mg day^{-1}) was not as rapid as the removal rate in the planted pot (Figures 2.5 and 2.6), and the contaminant losses for this no-plant treatment likely reflect natural attenuation (volatilization, sorption, etc.) and biodegradation of the compounds. However, the difference in concentration between the plant (Eucalyptus or Pine) and no-plant treatments is due to the presence of the trees, suggesting an enhancement of compound removal by the plants (phytoremediation effect), as presented in Table 2.2.

We were unable to detect chlorobenzene and benzene in the sand and plant roots, suggesting they were not bound to sand particles or root surfaces, but they might be possibly removed by plants and/or lost by natural attenuation and microbial degradation. The detection limit was 1 ug of compound per g of material (sand, root or leaf). Given a total amount of 200 mg of CB or BZ added to each pot throughout the entire experiment, there can be no more than 2.1 ± 0.6 mg of contaminants remaining in each pot (according to the detection limit). In addition, we sampled sand and roots from top and bottom parts of the pots to test any differences in contaminant concentration between the sand and roots that were immersed in contaminant solution (bottom) and the fraction not in contact with contaminant solution (top), although there was no detection of any compound on sand and roots for either one.

Furthermore, the non-detection of chlorobenzene and benzene in the Eucalyptus and Pine foliage suggests that these plants were not storing any of these contaminants inside their leaf tissues; thus, if the contaminants were taken up, they were either

metabolized inside plant tissues or release by plants to the atmosphere, although we did not determine the individual phytoremediation mechanism for chlorobenzene and benzene in our experiment. However, Burken and Schnoor (1998) were able to ascertain benzene uptake through plants and subsequent released from the leaves to the atmosphere because they used ^{14}C-labeled benzene, and Gomez-Hermosillo et al. (2006) ascertained a significant uptake of chlorobenzene by plants because they used ^{14}C-labeled chlorobenzene.

Eucalyptus exhibited a higher growth rate than Pine during our experiment (Figure 2.8), and this likely impacted the mass of contaminants in the pots. As a consequence, there is a slightly higher amount of contaminant (in mg) removed by Eucalyptus than Pine, although not significant (p-value > 0.12), mainly because Eucalyptus has a higher transpiration rate than Pine (p-value < 0.01), and there was more physiological activity by Eucalyptus than Pine.

In the no-plant treatment, the losses of both chlorobenzene and benzene occurred possibly because of either volatilization and/or microbial degradation (compounds used as carbon source). The volatilization potentially occurred because the top of the pot was open and the soil matrix was washed sand, which are two factors favorable for volatilization. Pure chlorobenzene has a vapor pressure of 12 mm Hg at 25^{o}C (Howard, 1991), a half-life of 21 days in the atmosphere, and a calculated half-life of 0.3 to 12 days in soil (Salgado and Marona, 2004). Benzene is even more volatile than chlorobenzene, with a vapor pressure of 95.2 mm Hg at 25^{o}C and half-live of 0.5 to 10 days in soil (Howard, 1991). Therefore, even without plants, these compounds would be lost anyway, but an important finding is that the use of plants enhances the loss rate of these

contaminants in soil/water. As a result, we are able to suggest the selection of *Eucalyptus urograndis* and *Pinus taeda* trees to enhance removal of chlorobenzene and benzene in field contaminated soils and groundwater.

2.5. Conclusions

Our greenhouse experiment suggests that *Eucalyptus urograndis* and *Pinus taeda* trees can be used for phytoremediation of soils and groundwater contaminated by benzene and chlorobenzene compounds. We were able to isolate the plant remediation effect, which was 5 to 50% of contaminant removal (in mass), from other potential losses like microbial degradation, volatilization, and sorption. Also, we have shown that plants can survive under high concentrations of chlorobenzene and benzene over many months of exposure, and visual/physiological observations from the greenhouse study confirmed that these plants can survive in contact with the contaminants, and even promote remediation. We did not find any contaminants in the sand or plant roots and foliage. Therefore, phytoremediation of chlorobenzene and benzene contaminated sites is a feasible and cost-effective technology for regions where *E. urograndis* and/or *P. taeda* are able to grow.

CHAPTER 3

POTENTIAL FOR PHYTOREMEDIATION BY *EUCALYPTUS UROGRANDIS* AT A SITE CONTAMINATED BY BENZENE-BASED COMPOUNDS IN SOUTHERN BRAZIL[1]

[1] Barcellos, D., L.A. Morris, T. Moura, D. Moura, and A. Thompson. To be submitted to International Journal of Phytoremediation.

Abstract

Contamination of industrial sites by volatile organic compounds is a problem worldwide. A former pesticide factory in southern Brazil has been contaminated primarily by chlorobenzene and minor co-contaminants including benzene, 1,2-dichloroethane, and 1,2-dichlorobenzene. In-situ chemical oxidation was initially applied to reduce the high contaminant concentrations to levels that could be polished using phytoremediation. Our primary research question concern whether or not Eucalyptus trees can affect contaminant movement and fate at this site in Southern Brazil. Hybrid *Eucalyptus urograndis* were planted at the site in July 2012 and within 1 year they grew an average of 4.0 m in height and 50 mm in diameter. The trees showed no mortality and they established well in soils and groundwater with contaminants and the chemical oxidant. No chlorobenzene or benzene was detected in the foliage and root tissues of Eucalyptus. Based on the groundwater results, we were unable to determine whether contaminant's remediation was occurring or not, and whether the remediation was due to phytoremediation or chemical oxidation. However, by using an evapotranspiration model, we estimated that the 200 m^2 Eucalyptus plantation has the potential to remediate 165 m^3 $year^{-1}$ of the contaminated groundwater within 1.5 to 5.0 m of depth (i.e., 55% of the volume of groundwater within 1.5 to 5.0 m of depth, in a year), and the potential to remove 4.53 kg $year^{-1}$ of chlorobenzene and 0.93 kg $year^{-1}$ of benzene. Additionally, soil and leaf analysis for nutrients shows an adequate balance of macro and micronutrients for Eucalyptus growth and for rhizodegradation of the contaminants by microbes. Therefore, phytoremediation may be used as an integrated remediation strategy, in combination with chemical oxidation, at this field site (RS, Brazil).

3.1. Introduction

Contamination of industrial sites by volatile organic compounds (VOC) is a global problem because of their substantial production and usage (Barro et al., 2009). Chlorobenzene and other benzene-based compounds are among the most common VOC contaminants in soils and groundwater worldwide (Lemaire et al., 2011; McCarty, 1993). Chlorobenzene, a monosubstituted aromatic compound (C_6H_5Cl), is moderately mobile in sandy soil and aquifers. This compound biodegrades very slowly in anoxic conditions, but biodegrades quickly in oxic conditions (USEPA, 2001). This pollutant represents multiple risks to human health and to the environment and may be toxic to humans (mainly in the central nervous system) when high levels are inhaled in drinkable water or encountered in the food chain (USEPA, 2000). The remediation of sites contaminated by these compounds can be challenging.

Among available remediation technologies, phytoremediation, the use of plants and their associated organisms to reduce or degrade pollutants in the environment (Schröder et al., 2007), is a relatively inexpensive and effective method for remediating certain contaminants. Phytoremediation projects can cost 50 to 90% less than physical, chemical and thermal remediation technologies (Rock and Sayre, 1998). Phytoremediation of contaminated sites can be accomplished by at least one of the following processes: rhizodegradation, phytoaccumulation, phytovolatilization, phytodegradation, and phytostabilization (Pilon-Smits, 2005). Compared to other organisms, plants have a higher survival when exposed to high concentrations of hazardous waste, and plants have the ability to degrade organic pollutants into harmless forms (Jones Jr et al., 2001).

According to Brigg's Law (Briggs et al., 1982), through the logarithm of the Octanol-Water Partition Coefficient (log K_{ow}), we can predict the fate of an organic compound when exposed to plants in the environment: log K_{ow} between 0.5 and 3.0 (moderate hydrophobic compounds) favors uptake, transformation and volatilization of organic compounds by plants; log $K_{ow} > 3.0$ (very hydrophobic compounds) favors adsorption on the roots allowing rhyzodegradation; and log $K_{ow} < 0.5$ (very hydrophilic compounds) does not favor uptake by plants (Schnoor, 1997). Chlorobenzene has a log K_{ow} of 2.98, which lies between a moderate and very hydrophobic compound. As a result, the primary removal processes for this compound are rhyzodegradation and phytovolatilization (Imfeld et al., 2009; Braeckevelt et al., 2011). On the other hand, benzene, which has a log K_{ow} of 2.13, is likely to be taken up by plants and can be either phytodegraded or phytovolatized (Burken and Schnoor, 1998; Nzengung, 2005).

In a chlorobenzene phytoremediation project, at a Superfund site in Baton Rouge, LA (USA), phytoremediation effectiveness was estimated by a mathematical model and compared with the general uptake equation from Burken and Schnoor (1998), and results showed an estimated plant uptake of 2.7 kg $year^{-1}$ ha^{-1} for chlorobenzene by willow trees (*Salicaceae* sp.) (Jones Jr et al., 2001). Also, buffering on the pollutant flux in the site was accomplished by the willow trees. Furthermore, there are projects involving phytoremediation of benzene using mostly hybrid poplar trees (McLinn et al., 2001; Green and Hoffnagle, 2004; Nzengung, 2005), and other phytoremediation projects for 1,2-dichloroethane (Green and Hoffnagle, 2004; Compton, 2003).

In addition to phytoremediation, plant systems can play an extra role in remediation of contaminated sites through absorption and transpiration of water in a

process termed phytopumping. Mature poplar trees (*Populus* spp.), for example, can transpire 200–1000 liters of water per day (Wullschleger et al., 1998). Phytopumping can be used to alter hydraulic gradients and form a hydraulic barrier to stabilize contaminant plumes and/or maintain an upward gradient to forestall downward contaminant leaching (Pilon-Smits, 2005). Therefore, plants are capable of providing a buffer zone and reducing the migration of contaminants downstream by uptake and rhyzodegradation of the contaminants (Nyer and Gatliff, 1996).

Trees of the Eucalyptus Family have the potential to be used as successful candidates in phytoremediation projects at sites contaminated by VOCs, due to their ability to adapt well in different environments, coupled with their fast growth and high transpiration rates. Several studies estimated that the evapotranspiration rate of mature *Eucalyptus* species lies between 3.0 to 10.0 mm day^{-1} (Dye, 1987; Facco, 2004; Sacramento Neto, 2001). Although Eucalyptus has been successfully applied for phytostabilization of heavy metals in Brazilian soils (Magalhaes et al., 2011; Melo et al., 2010), no studies using Eucalyptus trees to successfully phytoremediate soil and water contaminated by VOCs are published. Meanwhile, our lab group recently demonstrated that *Eucalyptus urograndis* can enhance the degradation of chlorobenzene and benzene compounds, when applied into soil columns under greenhouse conditions (Chapter 2).

A site located in southern Brazil (state of Rio Grande do Sul – RS) is contaminated by several benzene-based compounds, including mainly chlorobenzene, but also benzene, 1,2-dichloroethane, and 1,2-dichlorobenzene. The primary remediation technology that was selected to clean up the site was in-situ chemical oxidation (ISCO), using sodium persulfate to destroy the aromatic organic compounds, in order to reduce

pollutant's concentrations and to degrade contaminants into less toxic substances. Sodium persulfate ($Na_2S_2O_8$) is a relatively new chemical oxidant for groundwater remediation and has shown better performance than other chemical oxidants such as potassium permanganate and hydrogen peroxide (Liang et al., 2011). The persulfate anion ($S_2O_8^{2-}$) has a high redox potential (E_0=2.01 V), which can be increased by adding a strong base, such as NaOH (Furman et al., 2010). However, the complete remediation of contaminated aquifers to the low levels required by regulators is not entirely feasible in most cases (Travis and Doty, 1990).

Our primary research question is whether or not Eucalyptus trees can affect contaminant movement and fate in the soil/water system at a site in Southern Brazil. The specific objectives of this study were the following: (i) Assess plant survival in contaminant and chemical oxidant exposure under field conditions; (ii) evaluate phytoremediation potential and growth of *Eucalyptus urograndis* in a chlorobenzene/benzene contaminated site; and (ii) measure the effects of *Eucalyptus urograndis* on water table level and water movement at the site in Brazil (i.e., evaluate phytopumping effect). To reach these objectives we assessed plant growth and nutrient availability in soil and plant tissues; evaluated the survival of trees that were exposed to persulfate in both greenhouse and field conditions; analyzed groundwater, plant leaves, and roots for VOCs; and finally conducted evapotranspiration and water balance studies.

3.2. Materials and Methods

3.2.1. Site Characterization & Overall Project Approach

The field study site is located in the metropolitan region of Porto Alegre (Rio Grande do Sul State, Brazil), at an altitude of 45m (Figure 3.1). The climate in this region

is Cfa, according to Köppen classification, corresponding to a humid subtropical climate with an annual average temperature of 19 °C, and an annual average precipitation of 1300 mm, which is evenly distributed throughout the year (Nimer, 1990).

The field site is a former pesticide manufacturing facility located in an upland terrace (old floodplain) containing a contaminant plume that is moving slowly from the quondam pesticide synthesis area towards a river (Figure 3.1). The contamination was mainly due to storage tank leaks, formulation processes, and disposal practices; the primary contaminant is chlorobenzene, but benzene, 1,2-dichlorobenzene, and 1,2-dichloroethane are also present and are contaminants of concern.

Contaminant remediation at the field site involved several procedures. First, areas of exposed contaminated soil were excavated and placed in open drying sheds where natural volatilization and exposure to aerobic environments occurred. However, areas with greatest contamination were under concrete pads at groundwater depths between 2 to 5 meters, primarily in the sandy layers of the soil. For these areas, excavation was not an option. Instead, in-situ remediation was conducted by ISCO using sodium persulfate. This chemical was injected into the core of the plume through a series of injection wells (Figure 3.1) in 2012 and 2013. The amount of sodium persulfate injected (concentration around 200 mg L^{-1}) was expected to reduce the contaminant concentrations in the plume from 100 mg L^{-1} to 1 mg L^{-1} (or even lower). Thus, phytoremediation efforts were designed to decrease pollutants' concentrations within the residual contamination following this chemical treatment, and also potentially reducing contaminant plume movement. This combined remediation strategy provided chemical oxidation upgradient

and phytoremediation downgradient (Figure 3.2). The activities conducted at the site are listed in Table 3.1.

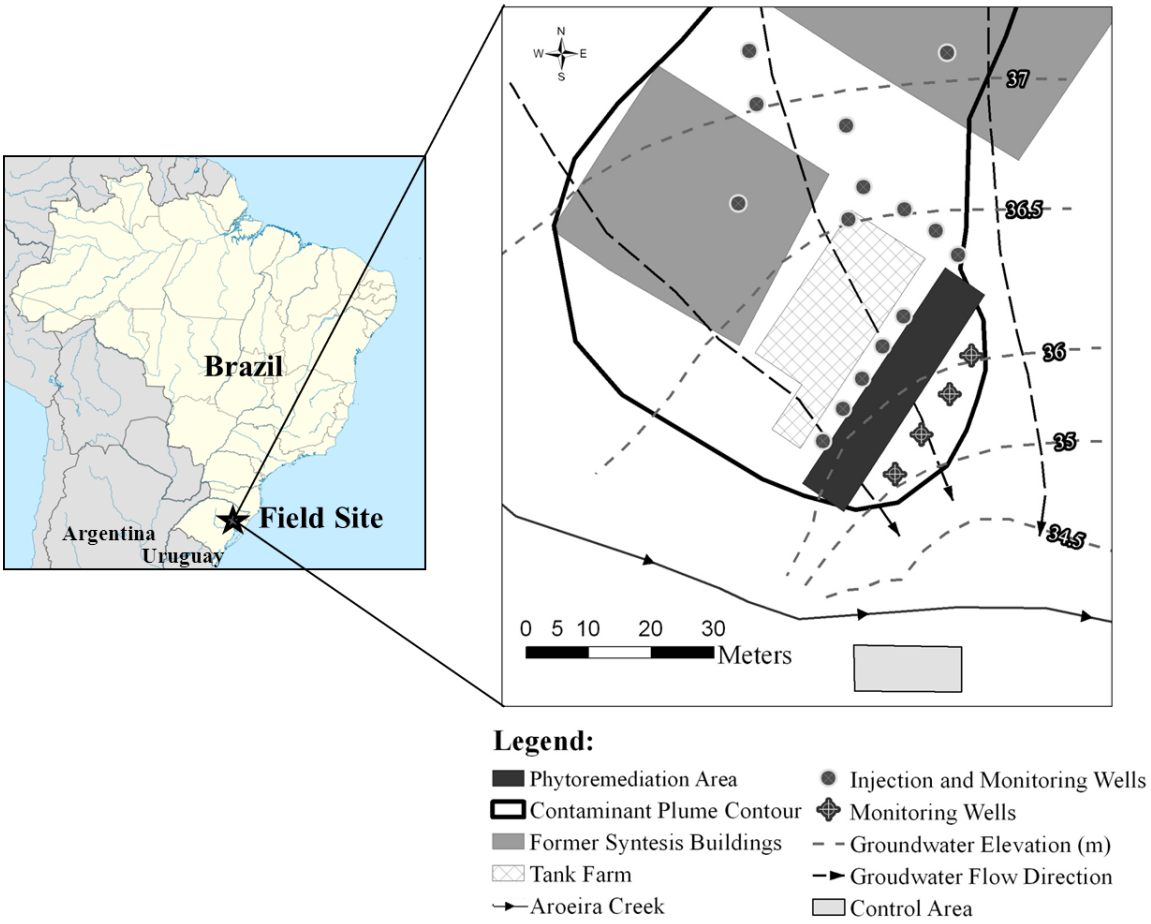

Fig. 3.1. Aerial view of the field site, in southern Brazil, showing contaminant plume, groundwater flow direction, Aroeira Creek, monitoring and injection wells, Phytoremediation area, and Control Area.

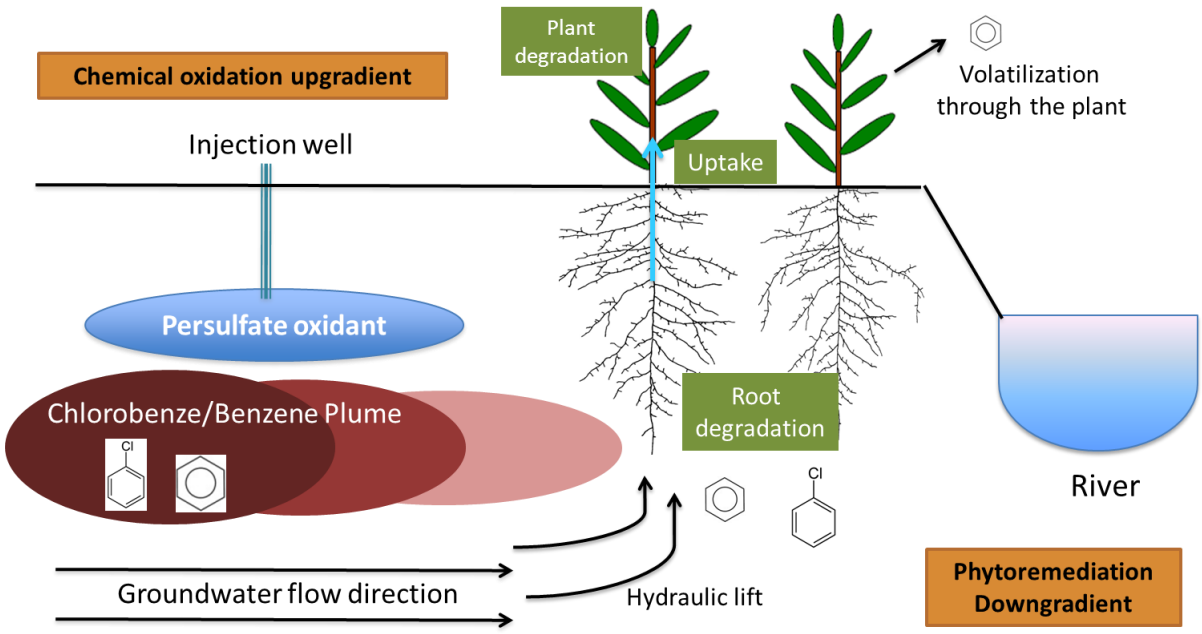

Fig. 3.2. Overall approach of the remediation project in field site: chemical oxidation upgradient and phytoremediation downgradient.

Table 3.1. Chronological sequence of primary activities at the field site

Activities	Dec-2011	Jul-2012	Sep-2012	Dec-2012	Jan-2013	Mar-2013	Jul-2013	Sep-2013
Groundwater sampling	x	x	x	x	x		x	x
Water table measurements†	x	x	x	x	x	x	x	x
Tree planting		x						
Sodium persulfate injections			x	x		x	x	
Tree measurements		x		x			x	x
Foliage and root for VOC analysis				x			x	
Soil and leaf for nutrients analysis		x		x			x	

† Measured almost bi-weekly from December 2011 to September 2013.

3.2.2. Establishment of the Phytoremediation Project

Preparing Planting Area & Soil Characterization

The area selected for phytoremediation was a rectangle plot of 36 m by 5.6 m (200 m^2), which was a former truck parking lot, located at the leading edge of the plume (Figure 3.1). Tree spacing was established as 1.8 m x 1.5 m, with a total of 75 seedlings planted in 3 rows (Figure 3.3). This area has a 20 cm thick layer of concrete above layers of compacted soils that were buried to flatten the area when the site was constructed. Additionally, we selected a control area (soil not contaminated), on the other side of the river (Figure 3.1), to compare results with the phytoremediation area.

The soils at the field site were in general very heterogeneous. Based on soil texture and drainage field observations, we divided the phytoremediation area into 3 Blocks (Figure 3.3): Block 1 in the South, Block 2 in the center, and Block 3 in the North. In the phytoremediation area, all Blocks (1 to 3) had a layer of mixed gravel and sand (15 to 20 cm thick) immediately below a concrete layer (20 cm thick). In addition, Block 1 had primarily a layer of dark red (2.5YR 3/6) clay loam (30cm) underlain a layer of pale brown (10YR 6/3) sandy loam (70 cm+), whereas Block 2 had stratified layers of grey (10YR 5/1) sand and dark red (2.5YR 3/6) clay loam (layers around 20-50 cm thick), and Block 3 had entirely a light red (2.5YR 6/8) clay soil just below the concrete and the gravel-sand layers. The original soils in the Control area had a top layer of dark-red (2.5YR 3/6) clay loam (from 0 to 50c m) that was followed by a layer of pale brown (10YR 6/3) sandy loam (50 cm+).

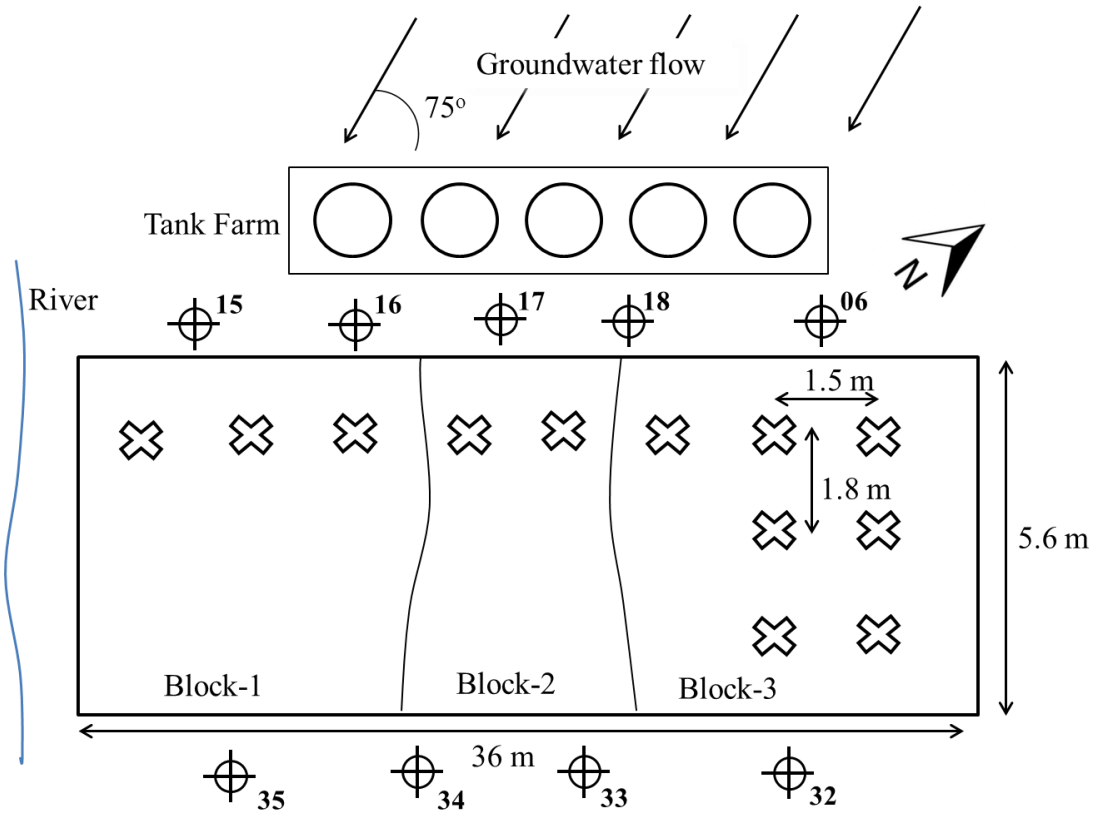

Fig. 3.3. Top view of the Phytoremediation Area, showing Blocks 1 to 3, space between plants, upgradient and downgradient wells (⊠ = Plants; ⊕ = Wells).

In the phytoremediation area, 75 holes of 50 cm in diameter by 1.5 m in depth were drilled into the concrete pad. Thus, concrete was left in the majority of the 200 m^2 area, except an area of 58 m^2 (75 individual holes) to plant seedlings. The original soil from each hole was completely removed and replaced with a new soil mixture, that consisted of 40% fine sand, 35% clay from a local soil (B horizon), and 25% chicken manure compost (% in volume). A characterization of the mixture components are presented in the Results section. Moreover, the mixture was prepared in a concrete mixer with increment of fertilizers (N, P, K, Zn, and B) and lime (Ca and Mg), following the dosage recommendation of Barros and Novais (1999).

Tree Plantings

The *Eucalyptus urograndis* (hybrid of *Eucalyptus urophylla* and *Eucalyptus grandis*) was selected to be planted at the site because of its high biomass production, formation of a deep and extended root zone, good adaptability in many environments, and high evapotranspiration rate, which are all excellent characteristics for phytoremediation and phytopumping purposes. Moreover, in our greenhouse study (Chapter 2), *Eucalyptus urograndis* was able to remove chlorobenzene and benzene from contaminated soil columns. The *E. urograndis* hybrids are widely planted in Brazil (including the region of Porto Alegre), have high biomass production (around 60 m^3 ha^{-1} $year^{-1}$) and strong disease resistance (Gomide et al., 2005). We planted seedlings (raised from seeds) that we purchased from local nurseries in the Porto Alegre region.

After soil preparation and placement, a seedling was planted in each hole of Blocks 1 to 3, and in the Control area. Plantings were completed in late July 2012, about 2 months before persulfate injections (see Table 3.1).

3.2.3. Trees Assessments

Plant Growth and Survival & Visual Analyses

The height and diameter of the Eucalyptus at field were recorded 2 to 3 times a year. Any visual and physiological problems with the trees were recorded, and any sign of mortality because of either contaminant exposure or persulfate exposure was recorded.

Plant Tissue Analyses for VOC and for Nutrients

Composited leaf samples were collected from at least 10 trees from each of the Blocks 1 to 3, and also from the Control area, for either VOC analysis or for nutrient

analysis. For VOC analysis, the composited leaf samples were stored in glass jars, placed in a cooler, and sent immediately to CEMIC laboratory (São Paulo, Brazil) for analyses by gas chromatography - mass spectrometer (GC-MS) according to an adaptation of the USEPA method 8260B (USEPA, 1996). We also sampled composited Eucalyptus roots (sampled at least 5 plants per Block) by digging lateral holes and collecting roots from 10 to 80 cm depth in the soil profile, trying to collect the roots in contact with the original soil (not the soil mix filler). Roots were also placed in glass jars, stored in a cooler, and analyzed for VOC in a GC-MS (also adapted USEPA method 8260B). The leaf and root samples were collected every 6 months.

For nutrient analyses of the composited leaf samples, N was determined by micro-Kjeldahl, while P, K, Ca, Mg, S, Cu, Zn, and Mn were analyzed in an Inductively Coupled Plasma Optic Emission Spectrometer (ICP-OES) after digestion in a mixture of nitric and perchloric acids, whereas boron was analyzed in the ICP-OES after dry digestion (Jones and Case, 1990; Bataglia et al., 1983).

3.2.4. Soil analyses (nutrients and texture)

Composited soil samples were collected for each of the 3 original soil types in the phytoremediation area and also for the original soils in the control area. Individual samples were collected for the components of the soil mixture: mineral soil (clayey B horizon), fine sand (commonly used for construction), and chicken manure compost. We also sampled the soil mixture after placement in the holes. Thus, a total of 12 soil samples were analyzed for nutrients and texture. Then, every 6 months (Table 3.1) we collected

soil and leaf samples from each Block and from the Control, to monitor the nutrient balance in the Eucalyptus plantation.

Soils were sent to the Laboratory of soil and plant analysis at the Universidade Federal do Rio Grande do Sul (UFRGS) in Porto Alegre (RS, Brazil). All soil samples were air-dried and crushed to pass through a 2 mm mesh sieve. The samples were analyzed for pH (1:1 soil:water ratio), particle size distribution (Gee and Or, 2002), total carbon by dry combustion (Nelson et al., 1996). Other elements (P, K, Ca, Mg, S, Zn, Cu, B, and Mn) were analyzed by atomic absorption spectroscopy, according to EMBRAPA (1997). The elements P and K were previously extracted with Mehlich-1 solution (Mehlich, 1953). The chicken manure compost, a component of the soil mixture filler, was analyzed with the same method used for leaf nutrient analysis (Jones and Case, 1990; Bataglia et al., 1983).

3.2.5. Groundwater Monitoring

Groundwater analysis for VOC & water table measurements

The wells NFMW 6, 15, 16, 17, and 18 are upgradient of the phytoremediation area, while the wells NFMW 32, 33, 34, and 35 are downgradient of the phytoremediation area (Figure 3.3). The sodium persulfate injections were conducted in all wells of the site, except NFMW 32, 33, 34, and 35 wells. Groundwater samples were collected every 2 to 6 months from the network of wells upgradient and downgradient of the phytoremediation area, and analyzed in a GC-MS for chlorobenzene and benzene, according to USEPA method 8260B (USEPA, 1996). In addition, variation in water table level was recorded by data loggers installed in the monitoring wells upgradient and

downgradient of the phytoremediation area, to verify effectiveness of phytopumping by Eucalyptus trees.

Estimate of Evapotranspiration for Eucalyptus

The Hargreaves equation (Eq. 1) (Hargreaves and Samani, 1985) was used to estimate the evapotranspiration from the planted trees in the Site. It required temperature, humidity, and latitude data from the local weather stations (Mantovani et al., 2007):

$$ETo = 0.0023\ Ra\ (Tmax - Tmin)^{0.5}\ (Tmean + 17.8) \qquad [Eq.\ 1]$$

Where, ETo = Potential evapotranspiration, in mm day^{-1}; Ra = Solar radiation at the top of atmosphere, in mm day^{-1}; Tmax = Maximum daily temperature (^{0}C); Tmin = Minimum daily temperature (^{0}C); and Tmean = Average daily temperature (^{0}C).

Temperature data were collected from 2 meteorological stations: one inside the site, and another located 13 km from the site (Davis Vantage Pro2 station). From August 2012 (right after planting of Eucalyptus) to October 2013, the temperature data were recorded from the two meteorological stations. Considering the field site's latitude south of 29.7^o, the values of Ra (solar radiation) were calculated from the Table B3 available in Mantovani et al. (2007). We estimated the Potential Evapotranspiration (ETc) for Eucalyptus, by multiplying the calculated ETo by the crop coefficient (Kc):

$$ETc = ETo \times Kc \qquad [Eq.\ 2]$$

The Kc value for Eucalyptus recommended by FAO/UN is 1.0 (Allen et al., 1998). This value of Kc = 1.0 for Eucalyptus has been previously used in Brazil (Silveira et al., 2012; Soares and Almeida, 2001; and Soares et al., 2001). However, for the initial

stage of the plants we used Kc= 0.7 during 3 months (Alves, 2009), then the Kc increased linearly for 10 months until it reached the value of 1.0, and stabilized at this value.

Water Balance

In order to estimate the annual water balance in the Eucalyptus plantation (at the Site), the water removed in the area was the Annual ETc subtracted by the Annual Precipitation, and the water entering in the area was the groundwater flow estimated by the One-dimensional Darcy flux equation:

$$q = K_{eff} \times (\Delta h/\Delta x) \qquad [Eq. 3]$$

Where K_{eff} is the effective saturated hydraulic conductivity (cm day^{-1}) calculated by the weighted average of the soil layer thicknesses on the saturated zone and their hydraulic conductivities (Radcliffe and Šimůnek, 2010), and by the gradient ($\Delta h/\Delta x$) of the area.

3.2.6. Potential contaminant degradation by Eucalyptus

Some organic compounds are taken up by plants at the same rate as they are available to roots in the soil solution. Typically, the plant uptake is adjusted by using a correction factor, which is the Transpiration Stream Concentration Factor (TSCF) that is correlated to the K_{ow} of the compound (Burken and Schnoor, 1998):

$$TSCF = 0.75 \exp(-(\log K_{ow} - 2.50)^2/2.4) \qquad [Eq. 4]$$

Assuming that log K_{ow} for chlorobenzene is 2.98, and log K_{ow} for benzene is 2.13, the TSCF for chlorobenzene is 0.68 and for benzene is 0.71. Retention by root surface has been shown for chlorobenzene, which can be accumulated on root surfaces (bounded

to lipids in cell walls) or can be taken up by plants, metabolized, and/or transpired from the leaves (Braeckevelt et al., 2008). On the other hand, benzene compounds can be removed from contaminated soil by plants through uptake process, since these compounds are hydrophobic enough to move through the lipid bilayers and soluble enough to travel into the cell fluid (Kamath et al., 2004).

Thus, binding or exclusion of the organic compound at the root interface decreases translocation and leads to lower TSCF values at log K_{ow} values greater or less than 1.8 (Burken and Schnoor, 1998). The general plant uptake equation is used with the term U for "uptake rate of the contaminant in mg day^{-1}" (Burken and Schnoor, 1998), but for this study the term Rem (stands for Remediation) is used because plants can either degrade chlorobenzene within the rhizosphere or take up and degrade it inside plant tissues. Thus, the modified potential remediation equation is:

$$Rem = TSCF \times T \times C \qquad [Eq. 5]$$

Where, Rem = Remediation rate of the contaminant, mg day^{-1} (instead of U, the uptake rate); TSCF = Transpiration Stream Concentration Factor, dimensionless; T = Transpiration rate of vegetation, L day^{-1}; and C = Compound concentration, mg L^{-1}.

3.2.7. Statistical Analysis

Statistical analysis of the data was performed using a one-way ANOVA and compared using the Tukey test for plant growth within blocks and between the phytoremediation and control areas, and also for groundwater results from wells up and down gradient. We used the SAS software (SAS Institute, 1995).

3.3. Results and Discussion

3.3.1. Trees Assessments

Individual holes were drilled into the concrete to plant trees because the alternative of using heavy machines to remove the concrete and to prepare the area for planting could induce desorption of contaminants into the soil solution, with the risk of contaminant movement downgradient towards the creek.

Regarding initial seedling adaptation, 2 to 5 weeks after planting, the Eucalyptus seedling mortality was around 20%, mainly because of the cold weather and hoar-frost during planting in southern Brazil (July 2012). The dead seedlings were replaced by new ones. After 3 to 5 months, there were only 3 plants that died (4% of the total of plants).

The height and diameter of the Eucalyptus in the phytoremediation area (Blocks 1, 2, and 3) and in the Control area are presented in Figure 3.4. No significant difference ($p>0.26$) in plant growth among the Blocks in the phytoremediation area was detected, and no significant difference ($p>0.11$) in plant growth between the phytoremediation (Blocks) and control areas was observed. Trees grew at an average of 0.35 ± 0.04 m per month (calculated from December 2012 to July 2013), with a few trees at 6.0 m tall and 70 mm in diameter within 1 year of age (Figure 3.5), especially considering that the soils where they are growing are very compacted, contaminated, oxidized, and have a concrete pad covering most of the area.

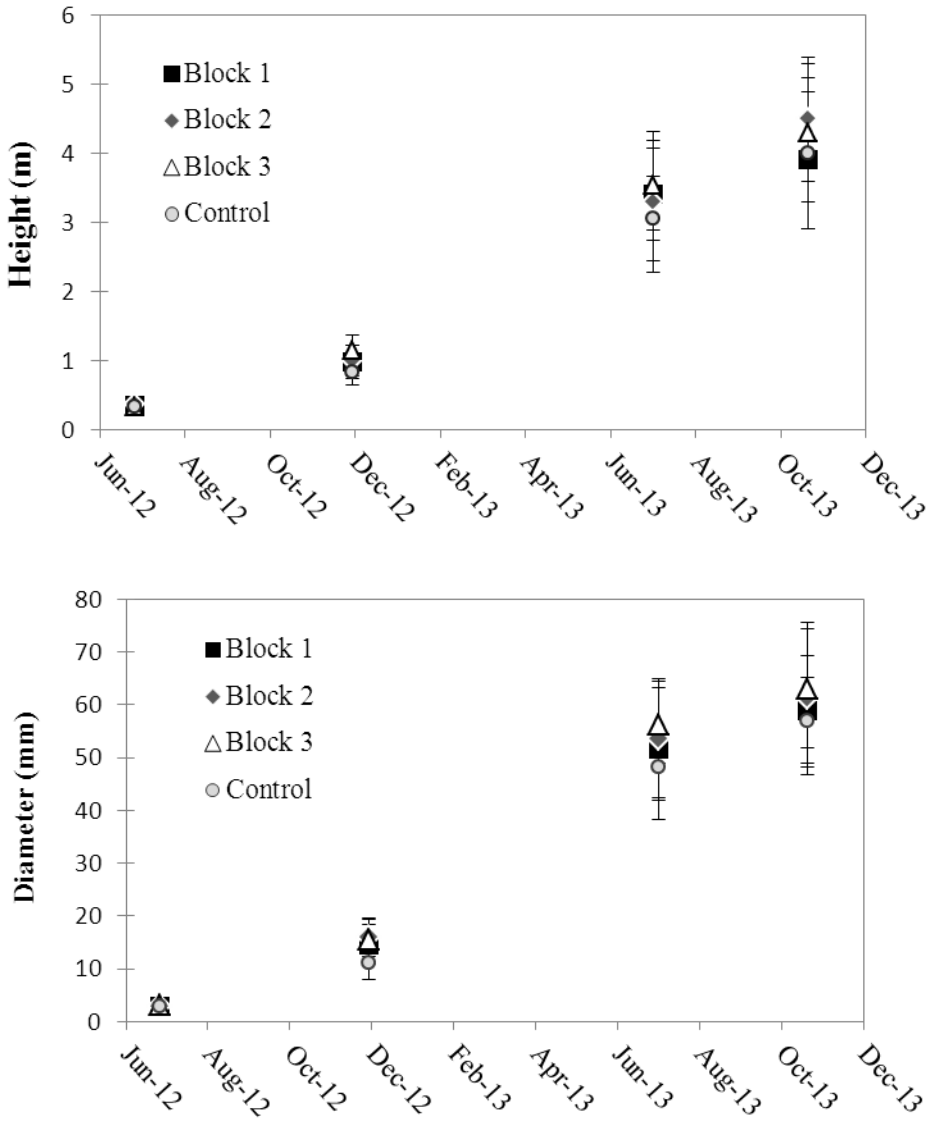

Fig. 3.4. Height and diameter of Eucalyptus trees at the field site ($n \geq 20$ reps) for the Blocks 1, 2, and 3 (phytoremediation area), and for the Control area (uncontaminated soil). Error bars are ± 1 S.D.

Fig. 3.5. Eucalyptus trees at the field site within age of 5 months (top) and 11 months (bottom).

Regarding exposure of Eucalyptus to the chemical oxidant (sodium persulfate), our greenhouse study (See Appendix A) demonstrated that *Eucalyptus urograndis* will die only if roots are completely immersed in a sodium persulfate solution at 10% concentration (either activated or not by NaOH). In the field site in Brazil, we observed a single plant (among the total of 75 plants) with leaves oxidized, possibly because of the persulfate injections. This plant was 1.0 m far from the surface of the injection well, where the persulfate solution (concentration of 200 mg L^{-1}) was injected until 2.0 m deep, upgradient of the Eucalyptus plantation. However, the plant that initially seemed to die

because of the oxidation effect of sodium persulfate, recovered after 1 month, and grew normally (6 months after initially affected by persulfate) (Figure 3.6). This observation confirms the hypothesis that plants would die only if the complete root system would be in contact with activated sodium persulfate at 10% concentration, as this plant in the field might have only the bottom of its roots in contact with sodium persulfate.

Fig. 3.6. Eucalyptus plant at site affected by sodium persulfate injection showing leaves oxidized (left) and the same plant recovered 6 months later (right).

As shown in Table 3.2, there was no detection for either chlorobenzene or benzene in foliage or roots of Eucalyptus in the phytoremediation area and in the control area. These foliage and roots results indicate that the plants are not storing chlorobenzene or benzene. The chlorobenzene can be degraded in the root zone (rhyzodegradation) or if taken up by the plants it can be metabolized inside the plant tissues (Braeckevelt et al., 2008). The benzene, if taken up by the plants, can be metabolized and/or volatilized through leaves (Burken and Schnoor, 1998). As a result, the Eucalyptus might be a pathway for the remediation of the contaminants reached by the root zone at the site.

Table 3.2. Results of VOC analysis (in ug kg^{-1}) for Eucalyptus foliage and roots at the site

	Date of Collection:	December 2012		July 2013	
	Compound	Foliage	Roots	Foliage	Roots
Block 1†	*Chlorobenzene*	N.D.*	N.D.	N.D.	N.D.
	Benzene	N.D.	N.D.	N.D.	N.D.
Block 2	*Chlorobenzene*	N.D.	N.D.	N.D.	N.D.
	Benzene	N.D.	N.D.	N.D.	N.D.
Block 3	*Chlorobenzene*	N.D.	N.D.	N.D.	N.D.
	Benzene	N.D.	N.D.	N.D.	N.D.
Control†	*Chlorobenzene*	N.D.	N.D.	N.D.	N.D.
	Benzene	N.D.	N.D.	N.D.	N.D.
Age of the trees (in months)		5		11	

† Blocks 1, 2 and 3 are the ones in the phytoremediation area, whereas the Control area is the uncontaminated soil.

* N.D. = not detected or concentration is below the detection limit of the GC-MS (0.5 ug g^{-1}).

The leaf nutrient analysis for the Eucalyptus at Phytoremediation area (Blocks 1, 2 and 3) and at Control area is described in Table 3.3. The following intrepretations are based on Dell et al. (2001), Malavolta et al. (1997) and Silveira et al. (2001) who provide an average of adequate leaf nutrient contents for the main Eucalyptus species planted in Brazil. We observed that all samples have the levels of N, Ca, Mg, S, Cu, Fe, and Mn in adequate concentrations within plant tissues, whereas the levels of P, K, Zn, and B are slightly higher than the adequate leaf nutrient range (Table 3.3). Another observation from Table 3.3 is that the levels of the nutrients P, Fe, Mn, and B are maintained about the same over 7 months of sample collection (December 2012 to July 2013), while the levels of N, Ca, Mg, S, Cu, and Zn showed reduction in the leaves for this period of 7 months, possibly because of availability reduction of these nutrients in the soil. Therefore, the Eucalyptus plantation maintains an adequate leaf nutrient content.

Table 3.3. Leaf nutrient analysis for the Eucalyptus (Total element content)

Samples	Collection Date	N (TKN)	P	K	Ca	Mg	S	Cu	Zn	Fe	Mn	B
		---------- dag kg^{-1} † ----------						---------- mg kg^{-1} † --------				
Block 1	Dec 2012	2.8	0.31	1.7	1.2	0.33	0.22	7	30	121	257	46
Block 2	Dec 2012	2.3	0.34	1.6	1.0	0.31	0.18	7	34	80	246	54
Block 3	Dec 2012	3.7	0.30	1.5	0.7	0.30	0.23	10	32	94	300	74
Control Area	Dec 2013	2.0	0.33	1.3	1.1	0.29	0.20	9	43	95	337	53
Block 1	July 2013	1.5	0.44	1.1	0.8	0.21	0.14	5	27	80	327	43
Block 2	July 2013	1.6	0.34	1.5	0.9	0.24	0.18	5	26	90	508	53
Block 3	July 2013	1.9	0.30	1.1	0.8	0.22	0.18	5	28	92	306	67
Control Area	July 2013	1.3	0.45	1.5	1.2	0.28	0.25	10	70	91	434	35

† The units in dag kg^{-1} and mg kg^{-1} express total element content.

3.3.2. Soil analyses (nutrients and texture)

As described in Table 3.4, soil-1, soil-2, soil-3 were the native soils from Blocks 1, 2, and 3, respectively, in the phytoremediation area before trees planting, and "Background" were 2 samples taken before tree planting in the area with uncontaminated soil (Control Area). The samples "sand", which is fine sand (used commonly for construction), and "clay", which is mineral soil from a local soil B horizon, are the previous components of the soil mixture. And, the sample "soil mixture" is the mixture of sand, clay, compost, fertilizers and lime that was placed in the holes before planting the trees. Then, the other soil samples were taken in the phytoremediation area (Blocks 1, 2, and 3) in December 2012 and July 2013 to monitor nutrient availability (Table 3.5). Additionally, Table 3.6 shows nutrients present in the chicken manure compost used in the soil mixture filler. The compost was added to provide nutrients for the plants, but also as a source of electron donors for pollutant biodegradation in the rhizosphere.

Table 3.4. Soil chemical analysis for original soils in the phytoremediation and control areas (field site)

	Depth	pH	P	K	SOM	Al^{3+}	Ca	Mg	Al + H	CEC	BS	S	Zn	Cu	B	Mn	Clay
Samples	cm		mg dm^{-3}		%	---------- $cmol_c$ dm^{-3} ----------					%	---------- mg dm^{-3} ----------					%
Soil-1	40-70	6.9	15	99	0.8	0	15	2.3	0.7	19	97	33	2.4	7.9	0.1	3	22
Soil-1	70-100	7.2	8.2	235	1.2	0	19	4.2	0.7	24	97	49	2.7	1.4	0.2	4	18
Soil-2	50-70	7.1	25	71	0.5	1.3	7.4	1.2	0.9	9.7	91	25	1.2	1.8	0.3	10	38
Soil-2	70-120	7.1	37	91	1.5	0.4	15	1.8	1.0	18	95	35	8.4	11	0.2	24	18
Soil-3	40-100	6.0	2.7	105	0.6	0	3.8	1.6	1.2	6.9	82	5.5	0.1	0.9	0.2	10	36
Background†	10-50	5.7	9.4	68	2.1	0	7.3	1.1	2.2	11	79	14	23	14	0.2	22	24
Background†	50-120	6.7	2.9	18	0.7	0	5.3	0.7	1.6	7.6	79	9.3	0.5	0.8	0.2	2	30

† Original uncontaminated soils, in the control area, before tree planting.

Table 3.5. Soil chemical analysis for soil mixture and its components, and soil nutrient monitoring in Dec-2012 and Jul-2013

	Depth	pH	P	K	SOM	Al^{3+}	Ca	Mg	Al + H	CEC	BS	S	Zn	Cu	B	Mn	Clay
Samples	cm		mg dm^{-3}		%	---------- $cmol_c$ dm^{-3} ----------					%	---------- mg dm^{-3} ----------					%
Soil Mixture and its components																	
Sand	-	4.9	4.1	12	0.5	0.6	0.2	0.1	2.2	2.5	13	20	0.1	0.3	0.2	2	6
Clay (B hor.)	-	5.0	4.2	23	1.0	1.3	2.5	1.4	7.7	12	34	73	0.6	1	0.4	14	44
Soil Mixture	-	6.7	>100	>400	2.3	0	8.3	3.3	1.2	16	93	227	35	0.7	1.3	6	19
Samples analyzed in December 2012 †																	
Block 1	10-60	6.6	>100	189	2.1	0	9.0	1.4	1.6	12	88	29	39	0.9	0.4	6	12
Block 2	10-60	6.7	>100	208	2.1	0	9.1	1.9	1.4	13	89	57	89	1.0	0.4	6	13
Block 3	10-60	6.6	>100	333	2.4	0	10	2.4	1.6	15	90	72	222	1.0	0.7	7	15
Control Area	10-60	6.1	>100	305	2.2	0	7.1	2.9	2.3	14	84	72	36	0.8	0.9	7	16
Samples analyzed in July 2013 †																	
Block 1	10-60	6.5	>100	248	2	0	5.8	2.4	1.6	10	85	19	50	0.6	0.6	4	13
Block 2	10-60	6.4	>100	357	1.8	0	6.5	2.3	1.7	11	85	56	56	0.5	0.8	2	13
Block 3	10-60	6.8	>100	>400	2.4	0	7.0	4.4	1.7	15	88	17	146	0.6	1.3	3	13
Control Area	10-60	5.8	>100	283	2.6	0	6.5	2.4	2.8	12	78	47	42	0.6	0.5	9	16

† Soil samples taken from the soil mixture filler (not the native soils) in Dec-2012 and Jul-2013, respectively.

Table 3.6. Nutrients in chicken manure compost (component of soil mixture for tree plantings)

	Humid	Total Content												
pH	Density	$C_{org.}$	N(TKN)	P	K	Ca	Mg	S	Fe	Na	Cu	Zn	Mn	B
	$kg\ m^{-3}$	---------- dag kg^{-1} ----------									------ mg kg^{-1} -------			
9	503	26	2	1.6	2.8	3.8	0.8	0.53	0.78	0.5	142	574	713	73

The following interpretations from Tables 3.4 and 3.5 were based on Alvarez V. et al. (1999) agronomic classification levels, who classified the nutrients availability in soils to plants as: very low, low, mean, high, and very high. The pH of the native soil-1, soil-2, and soil-3 was high to very high, while the pH in the Sand and Clay samples was low. But, after preparing the soil mixture, the pH reached a mean value, and stayed around the same value in December 2012 and July 2013 (for Blocks 1 to 3, and Control). The soil organic matter was low in the native soils, but in the soil mix and subsequent samplings, it reaches the mean level, given the addiction of the chicken manure compost. The nutrients P and K were in the mean level before planting, and reached very high amounts of availability to plants after Eucalyptus planting. Values of CEC, Ca, Mg, and BS (Base Saturation) were high in the native soils, in the soil mixture, and in all samples taken after planting (December 2012 and July 2013). There was some acidity (Al^{3+}) in soil-2, sand, and clay (B horizon) that was eliminated by mixing the lime into the soil mixture. The very high value of S present in the soils collected on December 2012 and July 2013 can be possibly because of the sulfur residuum from the sodium persulfate ($Na_2S_2O_8$) treatment within the phytoremediation area. Values of micronutrients are all in mean to high levels of availability for plants (Tables 3.4 and 3.5). The nutrients of the compost used for the soil mixture (Table 3.6) are all in high to very high levels, which is

good for the plants. Therefore, any fertility problem that existed in the soil was corrected on planting, or by adding extra fertilizers and/or compost later.

The adequate levels of nutrients in soils in the phytoremediation area are also good for microbial growth, which are also responsible for degrading organic pollutants. The Eucalyptus plantation in phytoremediation and control areas were fertilized every 6 months with macro and micronutrients following guidelines of Barros and Novais (1999).

The texture of the original soils from the field site, where Soil-1, Soil-2, and Soil-3 are the original soils sampled in Blocks 1, 2 and, 3, respectively, is presented in Table 3.7, as well as the texture for the soil mixture and its components. These laboratory texture results are similar to our field texture test (section 3.2.2), which illustrates the heterogeneity of soil texture within the layers of sand and clay distributed in the area. The fact that the soil mixture is a sandy clay loam favors root developing.

Table 3.7. Soil texture analysis for original soils at the Site and for soil mix and its components

Samples	Depth cm	Coarse sand	Fine sand	Silt	Clay	Textural Class
		---------- % ----------				
Soil-1	40-70	38	26	14	22	sandy clay loam
Soil-1	70-100	38	34	10	18	sandy loam
Soil-2	50-70	27	11	24	38	clay loam
Soil-2	70-120	36	33	13	18	sandy loam
Soil-3	40-100	29	22	13	36	clay loam
Sand †	-	29	64	1	6	sand
Clay (B hor.)*	-	20	25	11	44	clay
Soil Mixture	-	16	58	4	22	sandy clay loam
Background §	10-50	35	29	12	24	sandy clay loam
Background §	50-120	32	25	13	30	sandy clay loam

† Fine sand for construction used in the soil mixture to fill the holes at the field site.

* Clayey mineral B horizon soil from the surrounding region.

§ Original uncontaminated soils, in the control area, before tree planting.

3.3.3. Groundwater Monitoring

The results of chlorobenzene and benzene in groundwater at the field site are shown in Tables 3.8 and 3.9, respectively.

We observed a significant difference (p-value < 0.01) in the average of concentration (for chlorobenzene or benzene) between the up and down gradient wells, for the days 12/4/2012 and 8/13/2013. However, we do not have data for the downgradient wells before tree planting or persulfate injections, to compare results. In addition, since the trees were planted within 1 to 2 months of the chemical oxidation injections, we cannot infer whether Eucalyptus or the chemical oxidant (persulfate) might be influencing contaminant removal.

The average of the last concentrations recorded (August 13, 2013), in the wells upgradient of phytoremediation area, were 28.0 mg L^{-1} for chlorobenzene and 5.5 mg L^{-1} for benzene. These values will be used for the potential plant remediation equations (Topic 3.3.4). As shown in Figure 3.7, water level fluctuates over the months for the different wells in the phytoremediation area. Until the last data recorded (April 10, 2013), there was not a significant reduction in the water table by the plants in the phytoremediation area, mainly because the trees are still young (around 1 year old). Higher drops in water table level are expected when trees reach full canopy (around 2 to 3 years).

Table 3.8. Chlorobenzene groundwater results from the wells at the field site

	Before Planting		After Planting					
Collection Date:	11/28/2011	7/18/2012	11/27/2012	12/4/2012	12/19/2012	1/16/2013	3/21/2013	8/13/2013
	---------- ug L^{-1} ----------							
Upgradient Wells								
NFMW 15	NA†	44000	68500	NA	65000	59000	NA	39200
NFMW 16	NA	25500	NA	55000	53500	46500	NA	57500
NFMW 17	NA	18000	NA	14000	19400	14100	NA	14400
NFMW 18	NA	21000	17000	17400	24400	28400	NA	19100
NFMW 06	15960	110000	12800	12600	14300	10500	NA	10000
Downgradient Wells								
NFMW 32	NA	NA	NA	5450	NA	NA	NA	NA
NFMW 33	NA	NA	NA	3160	NA	NA	NA	11900
NFMW 34	NA	NA	NA	NA	NA	NA	5300	7
NFMW 35	NA	NA	NA	NA	NA	NA	6900	7600

† NA = not analyzed

Table 3.9. Benzene groundwater results from the wells at the field site

	Before Planting		After Planting					
Collection Date:	11/28/2011	7/18/2012	11/27/2012	12/4/2012	12/19/2012	1/16/2013	3/21/2013	8/13/2013
	---------- ug L^{-1} ----------							
Upgradient Wells								
NFMW 15	NA†	4300	10200	NA	9000	9240	NA	5300
NFMW 16	NA	6000	NA	10600	11000	9100	NA	7200
NFMW 17	NA	4500	NA	3500	4050	3900	NA	3600
NFMW 18	NA	8000	6100	6000	8000	7850	NA	5900
NFMW 06	103	760	7700	7800	8250	6000	NA	5600
Downgradient Wells								
NFMW 32	NA	NA	NA	1050	NA	NA	NA	NA
NFMW 33	NA	NA	NA	1580	NA	NA	NA	800
NFMW 34	NA	NA	NA	NA	NA	NA	840	0
NFMW 35	NA	NA	NA	NA	NA	NA	2200	2900

† NA = not analyzed

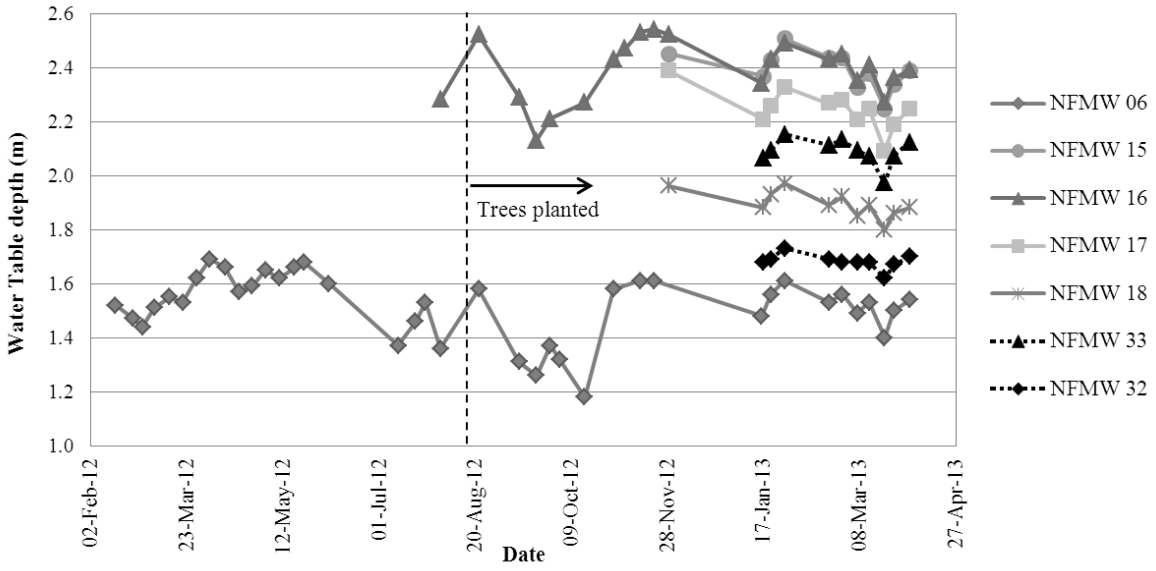

Fig. 3.7. Water table fluctuation (from surface) for wells upgradient (06, 15, 16, 17, and 18) and downgradient (32 and 33) of the phytoremediation area. No data recorded for wells 34 and 35.

Evapotranspiration

There are several methods to determine evapotranspiration (ET) of water from areas with a vegetative cover. These methods include: (i) measurements of soil moisture change such as by TDR, (ii) direct measurement of plant transpiration and surface evaporation, and (iii) estimates based on meteorological data. Estimation of evapotranspiration from meteorological data is the most commonly method used to calculate the reference evapotranspiration (abbreviated as ETo) for plants. There are many equations to estimate ETo, but Penman-Monteith is considered the standard one (Allen et al., 1998); however, this equation requires a lot of meteorological data. The Hargreaves equation (Eq. 1) (Hargreaves & Samani, 1985) is also commonly used worldwide, including Brazil. It only requires temperature, humidity, and latitude data (Mantovani et al., 2007). Therefore, we selected Hargreaves equation to estimate the

potential evapotranspiration of the Eucalyptus plantation, using the available meteorological data from the local weather stations, and we used Kc varying from 0.7 to 1.0 (Table 3.10).

Table 3.10. Calculation of ETo and ETc for the site of study

Year	Month	Rain	Tmean	Tmax	Tmin	Ra	Eto	Kc	ETc
-	-	mm	---------- ^{o}C ----------			mm day^{-1}	mm mo^{-1}	-	mm mo^{-1}
2012	Aug	52.2	19.6	26.3	14.6	9.87	89.3	0.70	62.5
2012	Sep	224.8	18.4	24.2	13.7	12.63	99.2	0.70	69.5
2012	Oct	130.2	21.6	27.4	17.3	15.23	133.6	0.70	93.5
2012	Nov	40.2	23.7	31.0	17.9	17.05	175.9	0.73	127.9
2012	Dec	210.8	26.1	32.5	20.8	17.82	188.8	0.75	142.4
									$\sum$ = 495.8
2013	Jan	94.6	26.1	32.5	20.8	17.50	185.4	0.78	144.9
2013	Feb	105.8	24.9	31.3	20.5	16.08	144.9	0.81	117.2
2013	Mar	119.8	21.7	27.2	17.5	13.93	119.7	0.84	100.1
2013	Apr	92.2	20.5	26.6	15.6	11.04	95.9	0.86	82.8
2013	May	69.6	16.6	22.1	12.2	8.69	65.8	0.89	58.6
2013	Jun	98.2	14.7	19.4	11.1	7.51	47.2	0.92	43.3
2013	Jul	123.2	13.7	20.2	9.0	7.96	58.8	0.95	55.6
2013	Aug	80.6	13.9	19.7	9.3	9.87	71.1	0.97	69.2
2013	Sep	122.2	17.8	23.2	13.2	12.63	92.2	1.0	92.3
									$\sum$ = 764.1

The annual potential evapotranspiration of Eucalyptus at the field (considering September 2012 to September 2013) is 1194 mm, which is reasonable for the region and is within the values described in the literature. Stape et al. (2010) conducted a multiscale experiment with Eucalyptus at eight sites across a 1000 km transect in Brazil, including the states: São Paulo, Minas Gerais, Espirito Santo and Bahia. They found a range of Potential Evapotranspiration (using Thornthwaite equation) from 876 to 1255 mm year^{-1} (average of 1117 mm year^{-1} for the eight sites). Almeida et al. (2007) monitored the

evapotranspiration of Eucalyptus plantations over six years and found a value 1092 mm $year^{-1}$ in Espirito Santo State, which has an average precipitation of 1147 mm $year^{-1}$. Also, Lima (1993) mentions that the annual evapotranspiration of Eucalyptus forests can reach a value of 1200 mm, which is a good representative value (Landford et al., 1980). Moreover, measurements of ET in the region of Concordia, Argentina, were recorded as 1150 mm for Eucalyptus, where the annual precipitation is 1352 mm (Nosetto et al., 2005). Therefore, the evapotranspiration value of 1194 mm $year^{-1}$ for Eucalyptus at the site seems to be in accordance with the literature.

From Table 3.10, we can also make the following assumptions: (i) Eucalyptus trees can transpire about 1194 mm $year^{-1}$, 100 mm $month^{-1}$, and 3.27 mm day^{-1} (given that 1194 mm per year = 1194 L m^{-2} $year^{-1}$); (ii) if the total area of Eucalyptus plantation in the site is 200 m^2, then the 75 plants can transpire 238,800 L of water per year, 19,900 L $month^{-1}$, and 654 L day^{-1}; and (iii) each individual plant may transpire 8.7 L per day. For the following years (2014, 2015, 2016, etc.), the ETc will be calculated using the Kc = 1.0, and ETc is going to vary according to the meteorological data, and if the plants remain healthy.

Water Balance

For the soil layer at 1.5 to 3.0 m depth at the site, the calculated K_{eff} (effective saturated hydraulic conductivity) is 10 cm day^{-1}, which approaches the K_s value for a sandy clay loam soil (Radcliffe and Šimůnek, 2010), which is the predominant soil texture in the area within 1.5 to 3.0 m depth. Furthermore, Table 3.11 shows the calculation for the water balance of the Eucalyptus plantation in the site. The ETc

(potential evapotranspiration) is considered as the plants have full canopy for the 200 m^2 that they cover, whereas the average annual precipitation of 1300 mm (Nimer, 1990) is considering only for the 75 holes (50 cm diameter) opened in the area for planting the trees, corresponding an area of 58 m^2.

Table 3.11. Water balance for Eucalyptus plantation, in one-dimensional view

Item	Calculation	Result
Water Removed:		
(1) Annual ETc in 200 m^2	(1200mm) x (200 m^2) =	240 m^3
(2) Precipitation in 58 m^2 †	(1300 mm) x (58 m^2) =	75 m^3
(3) Water balance (m^3)	(1) - (2) =	165 m^3
(4) Water balance (m)	(3) / 200m^2 =	0.83 m / year
Water Entering:		
(5) One-dimensional Darcy flux§	(10 cm/day) x (0.02) x (365 days) =	0.73 m / year
Annual net water balance	(5) - (4) =	- 0.10 m / year

† 58 m^2 is the area for the 75 roles of 50 cm I.D.
§ q= K_{eff} x Gradient (one-dimensional view, X-axis).

From Table 3.11, the ETc is slightly greater compared to the groundwater flux in the one-dimensional water balance approach. Thus, in one year, the water table may drop 10 cm. Nevertheless, in a daily basis, during the day the plants remove water from the unsaturated zone, but capillarity draws water up from the groundwater zone to replenish this water. As a result, on an annual basis the trees will not drop the water table significantly; however, it will be drawing in contaminated groundwater to the root zone of the trees (Figure 3.8). Indeed, for this particular area, the concrete favors the phytoremediation of the Eucalyptus because it induces the roots to grow down to the capillary fringe, as the annual net water balance is negative, due to the rain that is mainly drained superficially by the concrete.

In terms of contaminant removal in the area, the trees have the potential to remove 165 m^3 $year^{-1}$ of contaminated water. The volume of contaminated water under the concrete is primarily within 1.5 and 5.0 m (top of the water table and bottom boundary, respectively), and it was calculated as:

Vol = Depth x Superficial Area x Porosity = (3.5 m) x (200 m^2) x (0.42) = 294 m^3

Therefore, the Eucalyptus plantation in field site could be able to remediate around 55% of the contaminated water within 1.5 to 5.0 m depth, during a year.

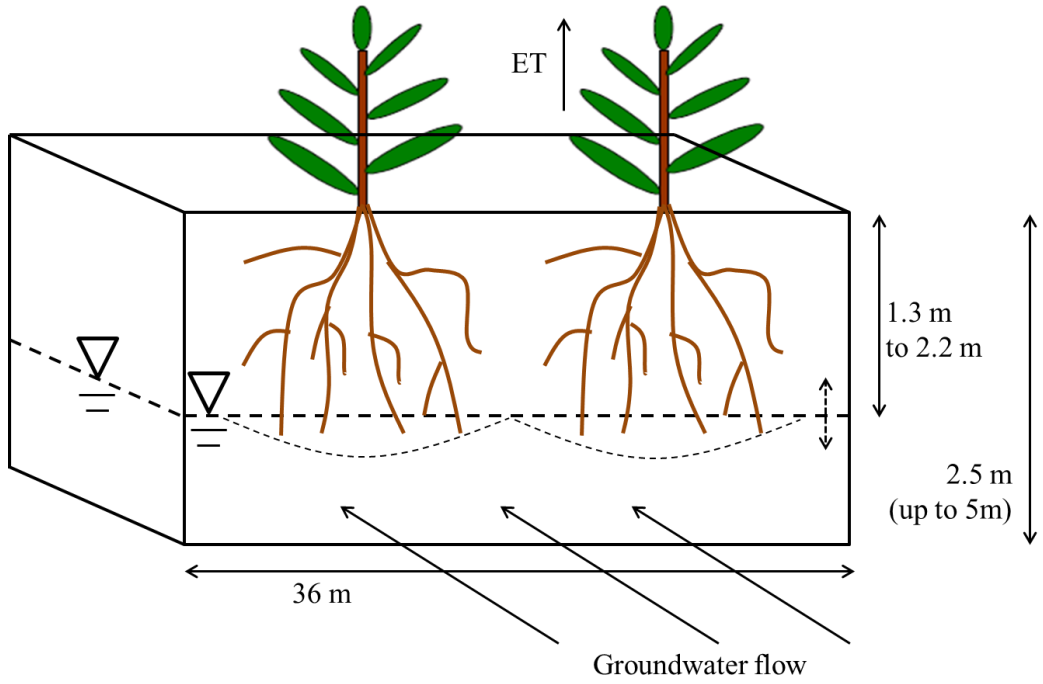

Figure 3.8. Illustration of the water table fluctuation at the site.

3.3.4. Potential contaminant remediation by plants

Using Equations 4 and 5 and the average contaminant's concentration last recorded (August 2013), we estimated the potential phytoremediation for chlorobenzene and benzene, as follows:

(i) For chlorobenzene:

Rem = TSCF x T x C = 0.68 x (8.7 L day^{-1}) x (28.0 mg L^{-1}) = 165.6 mg day^{-1} per plant. Thus, for the 75 plants in the site there will be a potential plant remediation of 12,423 mg day^{-1}. In a year it will be 4.53 kg of chlorobenzene potentially degraded by Eucalyptus. These values combined potential root/microbial degradation, plant uptake, and plant degradation.

(ii) For benzene:

Rem = TSCF x T x C = 0.71 x (8.7 L day^{-1}) x (5.5 mg L^{-1}) = 34.0 mg day^{-1} per plant. Thus, for the 75 plants in the site there will be a potential plant remediation of 2,550 mg day^{-1}. In a year it will be 0.93 kg of benzene potentially degraded by Eucalyptus. These values represent potential plant uptake, degradation, and volatilization of benzene.

In comparison, our greenhouse study using *Eucalyptus urograndis* showed a phytoremediation rate of 4.65 mg day^{-1} for chlorobenzene and 2.07 mg day^{-1} for benzene. The values calculated by the Equation 4 (for the field) and in the greenhouse study are different because the trees in the field are larger and are considered with full canopy (about 7 to 12 m in height) while in the greenhouse the experiment was conducted using small trees (around 1.2 m tall), which have evapotranspiration rates significantly lower. The measured ETc in the greenhouse was 0.55 L day^{-1}, which is 6.3 % of the field evapotranspiration rate (8.7 L per day). Supposing that the greenhouse plants could transpire 8.7 L day^{-1}, the phytoremediation rates would be: 71.9 mg day^{-1} for chlorobenzene and 32.0 mg day^{-1} for benzene. Now, it is possible to compare greenhouse and field phytoremediation processes.

Independently of these potential remediation rates, the Eucalyptus trees planted in the field experiment are likely inducing bacteria growth, increasing organic matter and root exudates in the soil, which should favor degradation of VOCs. The trees also reduced soil moisture under the concrete pad, which favors volatilization of VOCs as they degrade faster in aerobic conditions (Field and Sierra-Alvarez, 2008). In addition, plants frequently exudate enzymes and acids that may reduce chlorinated compounds (like chlorobenzene, 1,2-dichlorobenzene, and 1,2-dichloroethane) into chloride ions, carbon dioxide, and water (Schnoor, 1997).

3.4. Conclusions

According to modeling equations, the *Eucalyptus urograndis* plantation (75 trees) has the potential to phytoremediate the organic pollutants in the field experiment (200 m^2), at rate of 4.53 kg $year^{-1}$ for chlorobenzene and 0.93 kg $year^{-1}$ for benzene. However, groundwater results for chlorobenzene and benzene are inconclusive to affirm that plants or the chemical oxidant are promoting remediation at the site, mainly because there is no data for downgradient wells before tree planting and before ISCO. But, our greenhouse experiment supports that *E. urograndis* are able to phytoremediate chlorobenzene and benzene in contaminated soils. Foliage and root analysis did not show accumulation of chlorobenzene and benzene in plant tissues from the field experiment. Additionally, our model suggests trees could treat around 55% (165 m^3) of the contaminated groundwater within 1.5 to 5.0 m of depth, during one year.

The trees were able to survive in the field site after injections of sodium persulfate into the upgradient wells (up to 2.0 m of depth); however, trees may die when the entire root zone is exposed to the activated sodium persulfate at a concentration of 10%,

according to our greenhouse experiment. Enough nutrients are available for plant growth and for bacteria and root degradation of the organic pollutants present in the Site. The *E. urograndis* trees survived in the contaminated soil at the site and grew at a rate of 0.35 ± 0.04 m per month. No difference in tree growth was observed within the phytoremediation area, and between the phytoremediation and control (uncontaminated soil) areas. We did not observe significant water table drawdown from the wells at the phytoremediation area possibly because the trees are still young (1 year). Higher drops in water table may be expected for mature trees (2-3 years or more). Our field study suggests that *Eucalyptus urograndis* has the potential to remediate a portion of the contaminants in this field site in southern Brazil.

CHAPTER 4

CONCLUSIONS

Successful phytoremediation projects require: (1) that plants survive and grow in soils with the expected concentration of contaminants and under soil conditions likely to occur at contaminated field sites, and (2) that concentrations of contaminants can be reduced in the presence of plants. Our greenhouse and laboratory studies demonstrated that *Pinus taeda* seedlings survived under high concentrations of chlorobenzene and benzene contaminants, but young *Eucalyptus urograndis* trees would die if the entire root system is immersed in a solution of activated sodium persulfate at 10% concentration, which is an expected concentration within areas remediated by oxidative treatment. Our field study (in RS, Brazil) also showed that *Eucalyptus urograndis* trees survived in compacted soils containing mixtures of organic contaminants and sodium persulfate under unfavorable conditions beneath a concrete pad with limited surface exposure.

One of the main findings of this study is that *Eucalyptus urograndis* and *Pinus taeda* were able to enhance the removal of chlorobenzene and benzene from 1 to 10 mg within 2 days, equal to a removal of 5 to 50% of the initial contaminants concentration of 50 mg L^{-1}. We were able to isolate the plant remediation effect from other contaminant losses (such as microbial degradation, volatilization, and sorption), by using a no-plant control. Although we could not isolate which phytoremediation mechanism predominates in this greenhouse experiment, published literature indicates that chlorobenzene is likely

removed by rhizodegradation and phytovolatilization, whereas benzene is probably removed by plant uptake and phytovolatilization.

Groundwater results from the field site in southern Brazil are not conclusive to indicate that Eucalyptus and/or ISCO are remediating the contaminants, although there is a significant difference in contaminant concentration between up and down gradient wells. However, using modeling equations, we estimate that our field experiment (an Eucalyptus plantation of 200 m^2) has the potential to remediate 4.53 kg $year^{-1}$ of chlorobenzene and 0.93 kg $year^{-1}$ of benzene, from contaminated groundwater. We did not detect either chlorobenzene or benzene on plant roots and in plant leaves at the site, suggesting that Eucalyptus is not storing/sorbing these contaminants. For plant growth, phytoremediation, and microbial degradation purposes, we found that we were able to establish an acceptable balance of macro and micronutrients in the soils at the field site.

We also estimated that the amount of water removed annually by evapotranspiration of the planted Eucalyptus is almost the same amount that laterally enters in the area by groundwater flow over one year. Therefore, phytopumping by Eucalyptus plants will not reduce the water table more than a few centimeters per year. However, from water balance equations, we can affirm that Eucalyptus plantation has the potential to remove 165 m^3 $year^{-1}$ (or 55% $year^{-1}$) of the contaminated groundwater from 1.5 to 5.0 m of depth.

In conclusion, we demonstrated that phytoremediation of chlorobenzene/benzene contaminated soils can be effective, but we were unable to quantify the phytoremediation effect under field conditions in Brazil other than to demonstrate that Eucalyptus trees can grow under unfavorable soil conditions and they may promote phytoremediation.

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APPENDIX A

SURVIVORSHIP OF *EUCALYPTUS UROGRANDIS* UNDER CHEMICAL OXIDANT (SODIUM PERSULFATE) EXPOSURE: A GREENHOUSE STUDY

Before planting trees in the site in Brazil already treated with sodium persulfate (concentration around 10%), we set up a greenhouse experiment (at the University of Georgia, USA) to assess the survivorship of *Eucalyptus urograndis* exposed to sodium persulfate under different concentrations. We conducted two subsequent applications of sodium persulfate in pots containing plants in washed sand substrate, and monitored the solution pH and any signs of plant mortality. The solution was applied through the bottom of the pots by a clear PVC pipe that was also used to monitor the solution level (Figure A.1). After the first sodium persulfate application, by opening the pot valve, the solution was allowed to withdraw from each pot by gravity; then, after 3 days, the second sodium persulfate application started.

For the first application, the persulfate solution reached half of pot height (around 500 mL), whereas for the second application the pot was completely filled with sodium persulfate solution (around 1.0 L). In order to improve the chemical oxidation performance of sodium persulfate, the solution must be activated with NaOH by raising the pH value to at least 12.0. Thus, the experiment was designed to expose plants to persulfate solutions under the concentrations of 10%, 1%, and 0% (D.I. water) of $Na_2S_2O_8$ (sodium persulfate) which were either activated (NaOH added to raise the pH) or not activated (NaOH not added). In addition, a non-plant pot (control) was also

exposed to an activated 10% sodium persulfate solution. Thus, there were 7 treatments with 3 replications each.

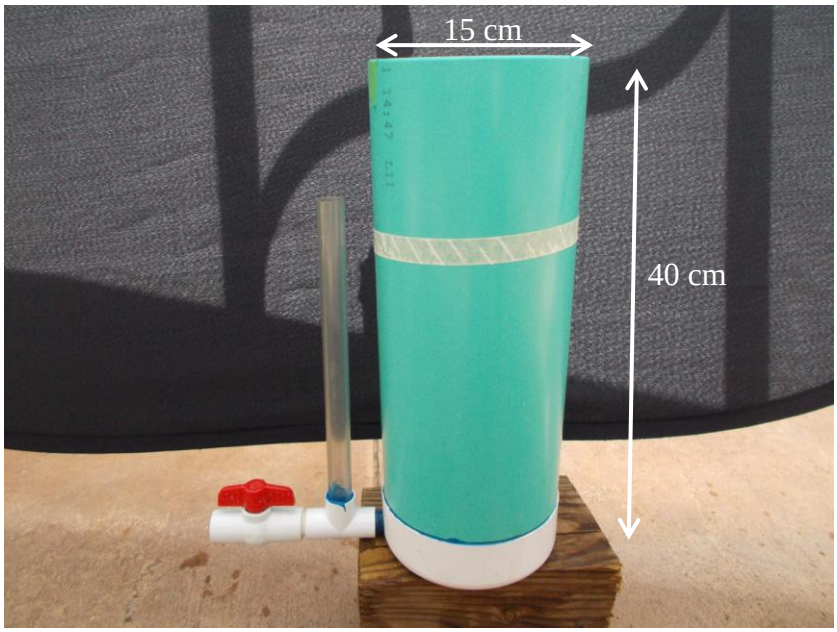

Figure A.1. Pot used for the chemical oxidantion experiment at greenhouse.

Given that plants transpired during the first and second sodium persulfate applications, as the water level dropped, fresh water was added every 4 to 8 hours until the initial water level was reached (half of the pot filled for the first application, and the pot completely filled for the second application). However, for the control treatment with no plant, no water was added as transpiration did not occur.

For the first application of sodium persulfate, there was no mortality observed on any treatment. As the first solution application reached only half of pot height, we did a second solution application exposing the entire plant root system by filling the pot

completely with solution. As a result, we observed mortality of *Eucalyptus urograndis* only for the treatment containing 10% of the activated sodium persulfate (treatment T1) a few hours after the application of the solution (Figure A.2). However, the treatment containing 10% of non-activated sodium persulfate (T2) does not show mortality for 2 days after application; but, on the third day, signs of mortality were evident for this treatment T2. The pH of the sodium persulfate solution was recorded in order to observe the behavior of the solution in the pots and to verify the possible effect on plants for the different treatments (Table A.1).

Figure A.2. Plant mortality after second application of activated sodium persulfate at 10% concentration.

As presented in the Table A.1, the pH on treatments T1 to T5 decreased rapidly for the first and second applications. On the other hand, for the control treatment (pot without plant) the pH did not decreased very fast, which shows that plants on the treatments T1 to T5 are also responsible for the quickly drop on pH, mainly by root

activities (acid exudates) and died roots exudates. The treatment T6 (Blank) confirms that Eucalyptus prefers to grow on an acidic environment: initial pH of 7.0 drops to around 5.5.

The alternation of treatments containing activated or non-activated sodium persulfate (addition of NaOH or not) was conceptualized to verify if the increase of pH would be the reason for plant mortality. But, results from treatments T4 and T5 (Table 6) demonstrated that high pH did not interfere with plant growth. The treatment T6, which is the blank treatment (only D.I. water added), was conducted to show that trees did not die because of the excess of water. Indeed, when the pots were completely filled with water (for the second application), the plants transpired all the water within 2 to 3 days. Also, the T5 treatment was conducted to verify if water at high pH (NaOH added) would either kill the trees or not.

In conclusion, only if *Eucalyptus urograndis* is completely immersed in the sodium persulfate solution at 10% concentration (either activated or not by NaOH), would rapid mortality be observed.

Table A.1. Values of pH over time for first and second applications of sodium persulfate (average for the 3 reps)

Treatments	**Persulfate Concentration**	**First Application of Persulfate**					**Second Application of Persulfate**				
		Hours					*Hours*				
		0	*6*	*30*	*80*	*100*	*0*	*24*	*48*	*60*	*72*
T1*	10 % Activated**	12.3	3.2	2.0	1.7	1.6	12.7	8.7	1.9	1.8	1.6
T2	10 % Non-Activated**	4.3	2.1	1.7	1.6	1.6	4.1	2.1	1.7	1.5	1.4
T3	1 % Activated	11.9	8.3	3.4	2.4	2.2	12.1	6.5	4.1	3.4	2.8
T4	1 % Non-Activated	5.5	2.4	2.1	2.0	1.8	5.8	3.2	2.7	2.3	2.1
T5	0 % (NaOH added)	12.7	12.4	12.2	10.3	10.1	12.8	11.9	10.5	9.8	9.5
T6 (Blank)	0 % (no NaOH added)	6.8	6.7	5.6	5.3	5.4	6.9	6.2	5.8	5.6	5.5
Control	10 % Activated	12.3	12.0	11.0	8.1	7.3	12.7	12.4	12.1	11.9	11.6

* Treatments T1 to T6 were pots containing plants, while the control treatment was a pot without plant

** Sodium persulfate activated (or not) by raising the pH with NaOH (or NaOH not added).