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Highlighted Article

Removal of 2,4-dichlorophenol in hydroponic solution by four *Salix matsudana* clones

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ABSTRACT

Using plants to treat polluted sites and groundwater is an approach called phytoremediation. The aim of the present study was to investigated the toxicity, uptake, accumulation, and removal of 2,4-dichlorophenol (2,4-DCP) in four *Salix matsudana* clones and to screen the feasibility of phytoremediation using *S. matsudana* clones. Willows were exposed to 2,4-DCP in hydroponic solution with the concentrations of 10, 20 and 30 mg L^{-1} for 96 h. The biomass of shoots and roots were reduced. Chlorophyll content decreased significantly compared with the control. All root morphology values were different between clones and different concentrations. The 2,4-DCP endurance of four *S. matsudana* clones was gauged as follows: clone 18 > clone 22 > clone 8 > clone 10. *S. matsudana* was found to promote 2,4-DCP removal relative to the contaminated solution without plants. From 52.2% to 73.7% of 2,4-DCP were removed by all treatments after 96 h exposure. 2,4-DCP was mainly accumulated in roots than in shoots. Clone 22 was the most efficient for the accumulation of 2,4-DCP in plant tissues. The removal of 2,4-DCP from the media may result from its degradation or polymerized in the root zone by the plant enzymes. Phytoremediation of 2,4-DCP with *S. matsudana* clone 8, 18 and 22 seem to be a viable option, especially at lower concentrations. These clones could remove 2,4-DCP from aquatic environment rapidly and efficiently. In addition, the toxic effect on trees during the removal process is not lethal.

1. Introduction

Chlorinated phenols represent one of the most abundant families of toxic industrial compounds. They are resistant to biodegradation and persist in the environment for long periods (Pera-Titus et al., 2004; Chaliha and Bhattacharyya, 2008). These pollutants come from the production of textile dyes, and pesticides, the bleaching of wood pulp, water chlorination, oil refining, and other common processes. Chlorophenols present a potential danger to human health owing to their carcinogenic, teratogenic, and mutagenic properties (Quan et al., 2004; Czaplicka, 2004). One of the most important members of this family is 2,4-dichlorophenol (2,4-DCP). Although its usage has been strictly restrained, there is still a great amount of 2,4-DCP contained in surface water, industrial effluents, wastewater, potable water, and soil as well as in atmospheric emissions from the combustion of municipal solid waste, hazardous waste, coal, wood, and herbicides (Laurenti et al., 2003; Talano et al., 2012). It has been recognized as a priority pollutant by the US Environmental Protection Agency and China's Environmental Protection Agency. Thus, different methods are needed for its removal.

Some physicochemical and biological methods for chlorophenols neutralization (including 2,4-DCP) have been reported. These include activated carbon adsorption (Shaarani and Hameed, 2011), chemical oxidation (Chaliha and Bhattacharyya, 2008), photocatalyst (Ogunbayo and Nyokong, 2011) and aerobic/anaerobic biological degradation (Dilaver and Kargi, 2009; Shin et al., 2010). But available physicochemical methods are expensive and not widely applicable. In addition, bioremediation (using microorganisms) is difficult to apply for practical remediation of pollutants at low concentrations. Enzymes have recently been used in many remediation processes to target specific pollutants for treatment (Zhang et al., 2009), but the enzymatic treatment has not been applied on large scale. In contrast to traditional physicochemical and biological methods, phytoremediation is a green technology in which plants are used to remediate soil, sediment, surface water, and groundwater environments contaminated by toxic metals, organics, and radionucleids. Phytoremediation has also the benefit of contributing to site restoration

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when remedial action is ongoing (Newman and Reynolds, 2004). Plant enzymes have been found useful for the decontamination of phenolic compounds in wastewater, where the detoxification effect was due to plant peroxidases (González et al., 2006). Phytoremediation has been shown to be an effective and economical way of treating recalcitrant contaminants, including chlorophenols, especially at low concentrations (Singh and Jain, 2003). Toyama et al. (2006) illustrated that aquatic plant (*Spirodela polyrrhiza*)-bacterial associations accelerated the degradation of 2,4-DCP. To date, few studies have evaluated the effects of plants on phenolic compounds. For example *Salix viminalis* exposed to 5 mg L^{-1} 2,4-DCP showed no signs of growth inhibition (Ucisik et al., 2007).

Willows have several traits that make them attractive for use in phytoremediation. These plants can accelerate the recovery of damaged ecosystems and re-establishing the natural ecological complex of organisms (Kuzovkina and Quigley, 2005). The effects of willows on organic pollutants has been studied (Vervaeke et al., 2003; Yu et al., 2007), but only few papers focused on the use of willows for the decontamination of 2,4-DCP (Ucisik et al., 2007). *Salix matsudana* is one of the most, native species in China, and they are phrearophytes and their roots are noted for their tolerance of flooded or saturated soils. In addition, willows has several characteristics that make it ideal for short-rotation coppice (SRC) systems including high yields, a broad genetic base, a short breeding cycle (Witters et al., 2009). The objectives of this study were (1) to determine the toxicity of 2,4-DCP to four *S. matsudana* clones, (2) to estimate the removal efficiency of 2,4-DCP and the accumulation of 2,4-DCP in *S. matsudana* clones, and in order to screen the feasibility of phytoremediation using *S. matsudana* clones to remove 2,4-DCP from wastewaters.

2. Material and methods

2.1. Plant materials

Four *S. matsudana* clones (clones 8, 10, 18, and 22) were obtained from Zhejiang Province. Tree cuttings 8 cm in length were removed from mature specimens. The cuttings culture and further experiments were performed in the greenhouse of the Research Institute of Subtropical Forestry, the Chinese Academy of Forestry. They were maintained in containers with modified Hoagland solution (KNO_3 0.51 g L^{-1}, $Ca(NO_3)_2$ 0.82 g L^{-1}, $MgSO_4 \cdot 7H_2O$ 0.49 g L^{-1}, KH_2PO_4 0.136 g L^{-1}, $FeSO_4$ 0.6 mg L^{-1}, H_3BO_3 2.86 mg L^{-1}, $MnCl_2 \cdot 4H_2O$ 1.81 mg L^{-1}, $ZnSO_4 \cdot 7H_2O$ 0.22 mg L^{-1}, $(NH_4)_6Mo_7O_{24}$ 0.45 mg L^{-1}, EDTA 0.744 mg L^{-1} and pH 5–6). To impede any algae growth, 10 mg L^{-1} $CuSO_4$ solution was added to all tanks (Coyner et al., 2001). The plants were acclimated to the solution under natural sunlight until new roots and leaves appeared. The solution was replaced twice a week to avoid nutrient shortage during the acclimation period. After 3 months of growth, uniform pre-rooted cuttings were transferred to polyvinylchloride containers (diameter 15 cm × height 15 cm) containing 1.5 L of sterile modified Hoagland nutrient solution and maintained until the beginning of experiments.

2.2. Tolerance tests

The responses of four *S. matsudana* clones to the toxicity of 2,4-DCP were determined. 2,4-DCP, purchased from Sigma-Aldrich with purity of 97% was used in this experiment. Four willow cuttings were transferred to a container which covered with a PVC septum (diameter 15 cm × height 15 cm, and 0.2 cm in thickness) with four holes drilled (2.5 cm in diameter), through which the plant shoots extend into the outside air space. The section of the shoots passing through the septum hole was wrapped with sponge sheets to minimize the open space. This design prevented the direct water evaporation from the nutrient solution into external air. The container was filled with 1.5 L of modified Hoagland solution with 2,4-DCP at the concentrations of 0 (control 1), 10, 20 and 30 mg L^{-1}. The experimental layout was a randomized block containing 3 replications of each treatment. The experiment was conducted for 16 h at 25–35 °C during the day and 8 h at 10–18 °C during the night. In order to block the light that might transform 2,4-DCP, the containers were covered with aluminum foil. In addition, 10 mg L^{-1} penicillin was added to Hoagland nutrient solution in order to eliminate microbe effects (Chapin et al., 1993). The initial pH of the solution was 5.6 and was not adjusted during the experiment. After 96 h of exposure, all plants were harvested to determine biomass, root morphology, and chlorophyll content.

The inhibition ratios of root fresh weight and shoot fresh weight were used to analyze the tolerance of willow clones to 2,4-DCP and were calculated as follows:

$$\text{Inhibition ratio } (\%) = \frac{W_1 - W_2}{W_1} \times 100\% \quad (1)$$

here W_1 refers to the fresh weight of roots or shoots of plants grown in control solution (without 2,4-DCP), and W_2 refers to the fresh weight of roots or shoots under various 2,4-DCP treatments.

The fine roots were freshly scanned by a root positioning system/STD4800 scanner (Regent Instruments, Inc., Canada) and root characteristics were analyzed with the WinRHIZO Pro 2005b (Regent Instruments, Inc, Canada). For spectrophotometric determination of chlorophyll concentrations, chlorophyll was extracted from leaf tissue (0.2 g) by homogenization in liquid nitrogen and subsequent threefold extraction with 80% (v/v) acetone. Twenty-four hours later, supernatants were used for analysis. Levels of the total chlorophyll was calculated using the extinction coefficients and the equations given by Hu et al. (2005)

$$\text{Total chlorophyll concentrations}(\text{mg ml}^{-1}) = 17.76_{A646.6} + 7.34_{A663.6} \quad (2)$$

2.3. Accumulation and removal of 2,4-DCP

The removal of 2,4-DCP from the containers was determined based on the difference between the initial 2,4-DCP mass and the final mass. At the end of the tolerance tests, the remaining 2,4-DCP in the nutrient solution as well as its concentration in roots and shoots were measured. Roots and shoots were rinsed with deionized water, sealed in separate bottles with gas-dense closures, and stored at −20 °C until extraction. In order to assess the 2,4-DCP removal from the growth solution not depending on plants, an abiotic control (control 2) without willow trees was run for each 2,4-DCP concentration in the same experimental conditions.

Mass balance is the mass (2,4-DCP) that entered an experiment system must, by conservation of mass, either leave the system or accumulate within the system.

Mathematically the mass balance for a system is as follows:

$$M_{\text{solution,initial}} = M_{\text{shoot}} + M_{\text{root}} + M_{\text{solution,final}} + M_{\text{removal}}$$

$$\text{Removal efficiency}(\%) = \frac{M\text{solution,initial} - (M\text{shoot} + M\text{root} + M\text{solution,final})}{M\text{solution,initial}} \times 100\% \quad (3)$$

where $M_{\text{solution,initial}}$ and $M_{\text{solution,final}}$ mean the initial and final mass, respectively of 2,4-DCP in the solution (mg). $M_{\text{shoot}} + M_{\text{root}}$ mean the mass of 2,4-DCP recovered from shoots and roots (mg). M_{removal} is the total mass of 2,4-DCP removed from the system (mg).

The root concentration factor (RCF), which describes the ratio of pollutant in roots relative to external solution used to assess the uptake capacity of four clones in the different level 2,4-DCP concentration. The RCF was calculated as

$$\text{RCF}(L\ \text{kg}^{-1}) = \frac{C_{\text{root}}}{C_{\text{solution}}} \quad (4)$$

where C_{root} (mg kg^{-1}) means 2,4-DCP concentrations in root at the end of the experiment, and C_{solution} (mg kg^{-1}) means the initial concentration in solution.

In addition, the shoot concentration factor (SCF, concentration ratio between shoot and initial solution) was calculated as

$$\text{SCF}(L\ \text{kg}^{-1}) = \frac{C_{\text{shoot}}}{C_{\text{solution}}} \quad (5)$$

where C_{shoot} (mg kg^{-1}) means 2,4-DCP concentrations in shoot at the end of the experiment, and C_{solution} (mg kg^{-1}) means the initial concentration in solution.

2.4. Chemical analysis

The concentration of 2,4-DCP in plant material and in Hoagland nutrient solution was measured by a gas chromatograph/flame ionization detector (GC-6890 N; Agilent, Santa Clara, CA, US) equipped with a low-polar capillary column of polyethylene glycol, model J&W DB-1701 (Agilent, Santa Clara, CA, USA), with a length of 30 m and an inner diameter of 0.32 μm. The temperature in the column varied between 90 and 190 °C. The carrier gas in the column was hydrogen with a flow of 30 ml min^{-1}. A 1 μl volume of sample was injected by an Agilent autoinjector 7683 at 250 °C. The peaks were analyzed with Agilent ChemStation B.01.02c solution. The standards for the calibration curve were prepared directly from the stock solution (20 mg L^{-1}). The detection limit was found to be 3.37×10^{-10} g. Aqueous samples were extracted within 2 min by dichloromethane four times with 25, 10, 10, and 5 mL, respectively. Shoots and roots were separately extracted at room temperature in Pyrex gas-dense bottles with stoppers containing the distilled water for 2 h by using an autoshaker (KYC-100C, Fuma, Shanghai, China).