

Uptake, Removal, Accumulation, and Phytotoxicity of 4-Chlorophenol in Willow Trees

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Abstract 4-chlorophenol (4-CP) is a well-known hazardous chlorinated compound and a precursor for the synthesis of the herbicide 2,4-dichlorophenoxyacetate. The relation between uptake, accumulation, toxicity, and removal of 4-CP in willow trees (*Salix viminalis*) was determined. In addition, the feasibility of implementing phytoremediation as a treatment method for 4-CP contamination was investigated. Willows were exposed to 4-CP levels ≤ 79.9 mg/L in hydroponic solution. The transpiration of the trees was used to determine toxic effects. Almost no inhibition of transpiration was detected at concentrations ≥ 15 mg/L. For concentrations ≥ 37.3 mg/L, transpiration decreased to $\leq 50\%$, and the trees wilted. Trees exposed to 79.9 mg/L wilted and eventually died. For concentrations of 79.9 mg/L, a significantly higher amount of 4-CP remained at the end of experiments in the test system compared with the amount remaining at all other concentrations. The loss of chemical from the system in experiments with trees was high, $\leq 99.5\%$. In treeless experiments, the mass loss of 4-CP was only 6% to 10%. The results indicated that degradation in the root zone is the main reason for the removal of 4-CP from the media. Phytoremediation of 4-CP in willow trees seems to be a remediation option, especially at concentrations < 37.3 mg/L, at which point degradation of 4-CP is rapid and efficient, and the toxic effects on trees are not lethal.

Chlorinated phenols are common environmental contaminants originating from wood-pulp bleaching, water chlorination, textile dyes, oil refineries, coking plants, and polymeric resins as well as chemical, agrochemical, and pharmaceutical industries. They are also used widely as biocides, fungicides, wood preservatives, and organic precursors of pesticides, such as 2,4-dichlorophenoxyacetate (2,4-D), 2,4,5-trichlorophenoxyacetate, and 2,4,6-trichlorophenol derivative (prochloraz) (Baggi et al. 2004; Clarkson et al. 1993). Chlorophenols are a threat to terrestrial and aquatic ecosystems because of their high toxicity, strong odor emission, high persistence in the environment, and suspected carcinogenesis and mutagenesis (Valli & Gold 1991; Mehmood et al. 1997; Quan et al. 2004; Tsuji et al. 2003; Oliver et al. 2003; Scraagg et al. 2003; Fahr et al. 1999; Kishino & Kobayashi 1994; Da Silva et al. 2003). Therefore, chlorophenols constitute a particular group of priority toxic pollutants listed by the United States Environmental Protection Agency in the Clean Water Act and by the European Commission 2455/2001/EC. One of the most important members of this chemical family is 4-chlorophenol (4-CP), a well known hazardous chlorinated compound and a precursor for the synthesis of the herbicide 2,4-D. 4-CP is released to the environment during the anaerobic degradation, followed by dechlorination, of polychlorinated phenols (Cole et al. 1994; Mikesell & Boyd 1986). Because 4-CP cannot be easily degraded under anaerobic conditions (Madsen & Aamand 1992; Woods et al. 1989; Häggblom & Valo 1995), it accumulates in the environment. Nevertheless, the main release of 4-CP is found at application sites of phenoxy acids and in wastewaters of the above-mentioned industries.

Different physicochemical and biologic methods have been proposed to treat chlorophenols, including 4-CP such as activated carbon adsorption, chemical oxidation, and

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aerobic and anaerobic biologic degradation in soil and water (Goswami et al. 2002; Kim et al. 2002; Wu & Kosaric 1991; Minero et al. 1995; Leonard & Lindley 1999; Jung et al. 2001; Hao et al. 2002; Danis et al. 1998; Loh & Wang 1998; Carvalho et al. 2001; Jianlong & Yi 1999; Bae et al. 1996). Available physicochemical techniques are expensive; do not yield full purification; and require a posttreatment process to remove the pollutant and undesirable byproducts from the contaminated environment (Goswami et al. 2002; Pera-Titus et al. 2004). However, biodegradation by conventional activated sludge systems is usually slow because of the inhibitory effect of chlorophenols on microbial metabolism (Pera-Titus et al. 2004; Toscano et al. 2003) and often fails to achieve high efficiency in removing chlorophenols from wastewater (Quan et al. 2004). Biodegradation by way of the direct application of adapted microorganisms capable of degrading chlorophenols can be another method for the practical remediation of pollutants. However, because bacteria are heterotrophs and need organic nutrients for growth and for degrading pollutants, the addition of nutrients to the polluted area is inevitable. This makes it difficult to apply a bacterial method for the practical remediation of pollutants at low concentrations (Tsuji et al. 2003).

High cost, incomplete purification, hazardous byproduct formation, and limited concentration-range applicability to traditional physicochemical and biologic treatments of contaminants have spurred the development of new remediation technologies. Advanced oxidation processes have been reported as one of the effective new remediation technologies for the degradation of chlorophenols from waters and soils, but they also involve great cost and require large amounts of reactants (Pera-Titus et al. 2004). Enzymes, such as peroxidases, have recently been used in many remediation processes to target specific pollutants for treatment. This treatment has many advantages with respect to other biologic or physicochemical methods: Handling and storage of isolated enzymes are easier than microorganism manipulation, and enzyme concentration is not simply related to bacterial growth. Moreover, conventional methods are not very selective, whereas the specificity of isolated enzymes is greater compared with other catalysts (Laurenti et al. 2003). In addition, insoluble polymers, which are formed during enzymatic removal, precipitate and can be separated by simple filtration or flocculation (Tong et al. 1997). However, enzymatic treatment has not been applied on a large scale, mainly because of (1) the high cost of enzymatic treatment and (2) the losses in enzymatic activity caused by adsorption of enzyme molecules on end-product polymers (Dec & Bollag 1994; Song et al. 2003). Finally, plant materials were found to be useful for the decontamination of phenolic compounds in water, during which the detoxification effect was caused by peroxidases contained in the plant tissue (Dec

& Bollag 1994; Durán & Esposito 2000). Roper et al. (1996) illustrated that the process of using horseradish roots as plant material to treat waters contaminated with phenols and anilines had broad substrate specificity and, therefore, a wide array of potential waste-treatment applications. Although plant materials, especially from agricultural wastes, can be used as an inexpensive enzyme source, no toxicologic test has been performed to ascertain whether or not plant materials release potentially hazardous compounds into the water. Moreover, significant amounts of plant material are required to treat the contamination, and their handling might be another environmental problem.

Plants and actinomyces can modify chlorophenols, often making them more soluble and easier to degrade by other microorganisms (Webb et al. 2001). Therefore, phytoremediation, which has been shown to be an effective and economic way of treating recalcitrant contaminants (Trapp & Karlson 2001; Singh & Jain 2003; McCutcheon & Schnoor 2003), might also be used to treat chlorophenols, especially at low concentrations, when bacterial degradation is not feasible. In this respect, the main questions are how 4-CP is removed and whether it is taken up into trees and causes toxic effects. The objectives of this study were therefore (1) to determine the toxicity of 4-CP to willow trees, (2) to estimate the removal velocity and mechanism of 4-CP, (3) to illustrate the relation between external and internal 4-CP exposure with respect to its toxicity to trees, and (4) to test the feasibility of implementing phytoremediation using willow trees as a treatment method for 4-CP in wastewater and soil.

Materials and Methods

Line of Experiments

Genetically identical willow cuttings (*Salix viminalis*) of similar size were grown in 500-mL Erlenmeyer flasks filled with modified International Organization for Standardization (ISO) 8692 nutrient (hydroponic) solution (ISO 1997) and exposed to 4-CP at varying levels. Transpiration of the trees was determined by weighing the flasks once a day for several days and used as the toxicologic end point. The possible role of 4-CP in root-zone degradation was also determined by using trees with detached leaves (stem with roots [leafless trees]) or detached roots (stem with leaves [rootless trees]). At the end of the experiment, the remaining concentrations of 4-CP in the nutrient solutions as well as its concentration in roots, leaves, and stems were measured to relate external to internal exposures and effects. To determine the bacterial degradation and volatilization of 4-CP in the system, additional experiments without trees were also performed.

Phytotoxicity Tests

The willow tree transpiration test for acute toxicity (Trapp et al. 2000) was applied to determine the short-term toxicity of 2,4-dichlorophenol (2,4-DCP) to willow trees. Reduction of transpiration was used as the end point to express toxicity.

The experiments were carried out in a fully climatized room with constant conditions ($26.8^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ and $30\% \pm 5\%$ relative humidity), under a rack of 36 W fluorescent lights, with a continuous light intensity of $54 \mu\text{mol}/\text{m}^2/\text{s}$. The racks were sequenced at intervals of 20 cm. Basket-willow cuttings with a length of approximately 40 cm were provided from Aage Bach (Tylstrup, Denmark) and pregrown for up to 5 weeks in buckets using tap water. Once the cuttings had well-developed leaves and roots, they were weighed and transferred into 500-mL aluminum foil-wrapped Erlenmeyer flasks with 150 mL ISO 8692 nutrient solution (pH 7.5), which were sealed with a cork stopper to avoid evaporation from the media. Further sealing of the flasks was done around the cuttings using plasticine (Modello Schulknete; Rudolf Reiser KG, Nürnberg, Germany). The trees were left for 2 days to adapt to the new environment.

After adaptation of the willows to the test conditions, transpiration was measured by weighing the flasks once a day to rank them. The trees were grouped based on their average transpiration, so that high- and low-transpiring willows occurred in every group containing five willow trees. Different 4-CP solutions (150 mL), which varied between 0 (control) and 80 mg/L, were added to these almost-identical groups based on transpiration. In other words, five replicates for each concentration were used in the toxicity tests. All concentrations were prepared with modified ISO 8692 nutrient solution to prevent nutrient deficiency from occurring during the test.

An additional set of experiments, in which roots were removed from the trees, was performed to determine the influence of 4-CP microbial degradation by associated bacteria located on and inside the roots. In another set of experiments, the leaves were detached to determine the effect of transpiration on the removal of 4-CP. A solution (150 mL) containing 7 mg/L 4-CP was added to the trees without roots and leaves.

Removal and Accumulation of 4-CP

At the end of the phytotoxicity tests, leaves, roots, and stems were rinsed with deionized water, sealed in separate bottles with gas-dense closures, and stored at -18°C until the extraction to determine the accumulation of 4-CP in willows. The final 4-CP concentration in nutrient solution

was determined immediately after the termination of the phytotoxicity tests.

To determine biotic and abiotic degradation losses of the compound, two sets of flasks with 4-CP solution were prepared without willow cuttings. The initial 4-CP concentration was 11 mg/L for both sets. The first set of samples was placed without willow trees in Erlenmeyer flasks sealed with plasticine as in the phytotoxicity experiments, whereas the second set of samples was put into glass bottles closed with gas-dense blue caps. Four replicates were used for each set of controls.

Calculation of Results

To compare the toxic effect of 4-CP on tree cuttings with different initial transpiration (before the addition of toxicant), transpiration was normalized with respect to initial transpiration and transpiration of the control cuttings. This was necessary because individual trees have different transpiration rates, and, concurrently, (healthy) trees grew during the test. Normalized relative transpiration (NRT) was calculated by:

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

where C is concentration of the compound in hydroponic solution (mg L^{-1}); t is time period (hours); T is absolute transpiration (g h^{-1}); and n and m are the number of replicates for exposed trees and control trees, respectively. The NRT of the controls was always 100%. Values of the treated trees $<100\%$ indicate inhibition of transpiration.

Effective concentrations (ECs) were also calculated based on the NRT at 72 hours. For calculation of EC values and confidence limits, a statistical programme using weighted nonlinear regression was employed. The programme assumes a logarithmic normal distribution of data, and for calculation of confidence limits it uses inverse estimation, taking into account the covariance within the control response (Andersen 1994).

The loss of 4-CP from the flasks was determined based on the difference between the initial and final 4-CP mass. The final 4-CP mass was the sum of remaining mass in the hydroponic solution at the end of test plus the mass recovered from the plant material.

Chemicals and Chemical Analysis

4-CP with a purity of 98% was purchased from Merck-Schuchardt (Munich, Germany). The concentration of 4-CP

in plant material and hydroponic solution was measured by a gas chromatographer–flame ionization detector (model GC-2010; Shimadzu, Kyoto, Japan) equipped with a non-polar capillary column of polyethylene glycol (model ZB5MS; Phenomenox, Torrance, CA) with a respective length and inner diameter of 30 m and 0.25 mm, respectively. The temperature in the column varied between 60°C and 240°C. The carrier gas in the column was hydrogen with a flow of 47 mL/min. A volume (2 µL) of sample was injected by Shimadzu autoinjector AC-20i at 300°C. Peaks were analyzed with GCsolution software (Shimadzu). Standards for the calibration curve were prepared directly from the stock solution (150 mg/L). The method used in this study was validated as previously described in the literature (Harris 2003). The detection limit was found to be 0.72 mg l⁻¹. Aqueous samples were extracted within 2 minutes by a solvent mixture of 49.8% (v/v) diethyl ether and 49.8% (v/v) pentane with 0.4% undecane-11 as internal standard. Plant materials were extracted at room temperature, in Pyrex gas-dense bottles with stoppers containing the same solvent mixture, for 48 hours using an autoshaker (Struers LS5 Shaker; Gerhardt, Berlin, Germany).

Results

Phytotoxicity of 4-CP to Willows

Figure 1 shows the NRT of willows grown in spiked hydroponic solution. Willows exposed to 6.8 mg/L 4-CP showed almost no sign of inhibition. At a 4-CP concentration of 14.5 mg/L, transpiration of the willow decreases only slightly lower than that of controls and totally recovered by 92 hours. The trees exposed to 37.3 mg/L 4-CP showed decreased transpiration of 45% after 92 hours. Finally, at a 4-CP concentration of 79.9 mg/L, the trees showed signs of wilting and almost stopped transpiring after 92 hours. The EC₁₀, EC₂₀, and EC₅₀ values were calculated based on the NRT of willows and are listed in Table 1. The EC₅₀ for willow trees in the toxicity test was 32.20 mg/L.

Mass Balance

4-CP was detected neither in plant nor in the remaining solution for the controls (0 mg/L). The mass balance of 4-CP for the willows exposed to the chemical is listed in Table 2. No accumulation in leaves or stems was detected for the given concentrations. Although it was clearly observed that 4-CP accumulated in roots, no significant differences in accumulation were found in the roots of trees

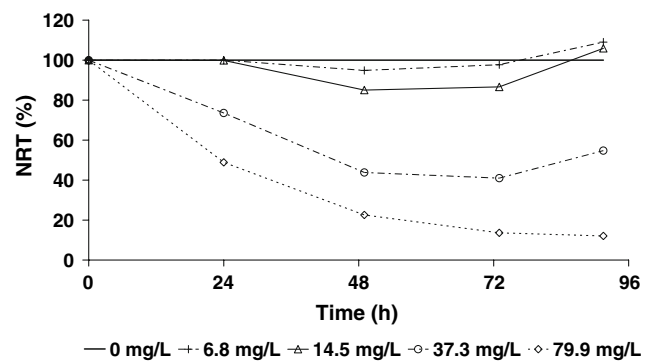


Fig. 1 NRT of willows (*S. viminalis*) grown in hydroponic solution at different 4-CP concentrations. Values represent the mean of five replicates

exposed to different 4-CP concentrations, as shown in Figure 2 (one-tailed Student *t* test; $\alpha = 5\%$). Average concentrations in exposed roots were in the range of 10 to 15 mg/kg. At the end of the experimental period, all of the final 4-CP concentrations were <1 mg/L, except for the tests performed with trees exposed to 79.9 mg/L initial 4-CP solutions. No significant difference in the losses was detected during the experiments ($\alpha = 5\%$). Almost all of the supplied 4-CP was removed.

The losses from the treeless samples in flasks closed with cork stoppers and those in flasks with gas-tight caps were 10% and 6%, respectively, for the samples spiked with 11.4 mg/L 4-CP. This shows that sorption to roots and microbiologic degradation might be responsible 6% of the loss and volatilization for additional 4% because volatilization may occur in the bottles closed with cork stoppers but not in bottles closed with gas-tight caps.

Loss of 4-CP from the media between the experiments performed with and without trees was significantly different (one-tailed Student *t* test; $\alpha = 5\%$). Willow trees significantly increased the removal of 4-CP compared with treeless experiments because almost all 4-CP in the hydroponic solution was removed for all examined initial concentrations (see Table 2), whereas only 10% loss was detected for the treeless experiments.

Table 1 ECs based on NRT at 72 hours

EC	mg/L	95% Confidence limits	
		Lower	Upper
10	12.0	5.46	26.50
20	16.90	9.18	31.00
50	32.20	22.60	45.90

Table 2 4-CP mass balance at 92 hours

C _{initial} (mg/L)	Solution initial (mg)	Leaves (mg)	Roots (mg)	Stems (mg)	Solution final (mg)	Total mass ^a (mg)	Loss ^a (%)	Loss ^b (%)
6.8	1.02	<0.02 (NA)	0.03 (0.02)	<0.45 (NA)	<0.04 (NA)	0.03	97.1	47.1
14.5	2.18	<0.02 (NA)	0.05 (0.03)	<0.48 (NA)	<0.03 (NA)	0.05	97.7	73.3
37.3	5.60	<0.02 (NA)	0.03 (0.03)	<0.71 (NA)	<0.06 (NA)	0.03	99.5	85.3
79.9	11.99	<0.02 (NA)	0.02 (0.02)	<0.62 (NA)	0.48 (0.42)	0.50	95.8	90.5

SDs are in parentheses. NA = SD not available because measured values were lower than the detection limit; solution final = final mass of 4-CP in the hydroponic solution; solution initial = initial mass of 4-CP in the hydroponic solution; total mass = total mass of 4-CP recovered from plant and solution

^a Values lower than the detection limit were considered as zero

^b Values lower than the detection limit were considered as the detection limit

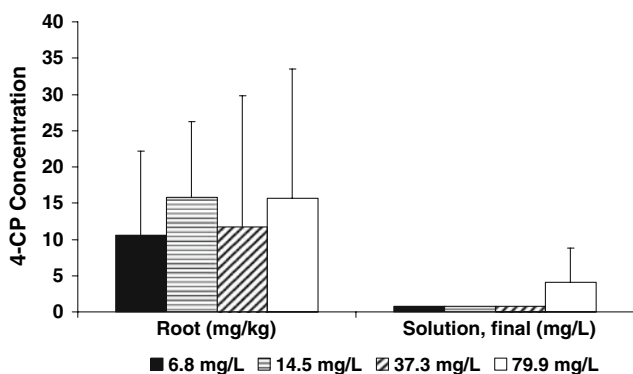


Fig. 2 4-CP accumulation (mg/kg) in roots and concentrations (mg/L) in hydroponic solution at the end of the experiment ($t = 92$ hours). Error bars = 95% confidence interval

Root Concentration Factor

The root concentration factor (RCF; in L/kg) describes the ratio between concentration in roots (mg/kg) and concentration in external solution (mg/L). Organic compounds may dissolve in the aqueous root sap or sorb to root lipids (Trapp 2002). Often, there is a linear relation, *i.e.*, the root concentration increases when the concentration in solution increases, and the RCF increases with increasing lipophilicity of the compound. This is described by the RCF regression from Briggs et al. (1982):

$$RCF = 0.03 \times K_{OW}^{0.77} + 0.82$$

Given the K_{OW} of 4-CP as 2.42 (Rippen 1991), an RCF of 3.01 L/kg results. This theoretical value, based on phase equilibrium between external solution and roots, can be compared with the experimentally determined RCF values (Table 3). Measured RCF values ranged from 0.2 to 1.55 for intact trees and decreased with increasing 4-CP concentrations in the solution. Throughout the experiments, actual RCF values were lower than the calculated RCF value. In the experiment with trees having

Table 3 Observed RCFs at 92 hours

C _{initial} (mg/L)	RCF (L/kg)
6.8	1.55
14.5	1.09
37.3	0.31
79.9	0.20
7.5 ^a	3.00

^a Leafless trees

detached leaves, an RCF value of 3.0 was found, which was close to the predicted RCF value.

Determination of Root-Zone Degradation

In the experiments using rootless trees, loss of 4-CP from the system was drastically decreased; the final 4-CP mass remaining in the nutrient solution at the end of experiments with rootless trees was 43.8% of the initial mass (Table 4). However, leafless willows showed no significant difference in remaining 4-CP mass in hydroponic solution from that of intact plants (Student t test, $\alpha = 5\%$). As expected, transpiration was lower when roots or leaves were detached (Fig. 3).

Almost all of the initial supplied volume (150 mL 4-CP) to rootless trees and leafless trees was still present at the end of experiment. The remaining volume was 138 (± 4) and 137 (± 2) mL for trees with detached leaves and roots, respectively.

Discussion

Comparison of 4-CP Toxicity to Willow Trees with Toxicity to Other Species

In the present study, 4-CP was found to be less toxic to willows than to aquatic plants, such as duckweed (*Lemma*

Table 4 4-CP mass balance with rootless trees and leafless trees at 92 hours

Treatment	C _{initial} (mg/L)	Solution initial (mg)	Leaves (mg)	Roots (mg)	Stem (mg)	Solution final (mg)	Total mass ^a (mg)	Loss ^a (%)	Loss ^b (%)
No leaves	7.45	1.12	No leaves	0.02 (0.02)	<0.39 (NA)	<0.10 (NA)	0.02	97.9	54.3
No roots	7.45	1.12	<0.02 (NA)	No roots	<0.21 (NA)	0.49 (0.13)	0.49	56.2	35.7
Intact plant	7.45	1.12	<0.02 (NA)	0.01 (0.01)	<0.30 (NA)	<0.06 (NA)	0.01	99.2	37.7

SDs are in parentheses. NA = SD not available because measured values were lower than the detection limit; solution final = final mass of 4-CP in the hydroponic solution; solution initial = initial mass of 4-CP in the hydroponic solution; total mass = total mass of 4-CP recovered from plant and solution

^a Values lower than the detection limit were considered as zero

^b Values lower than the detection limit were considered as the detection limit

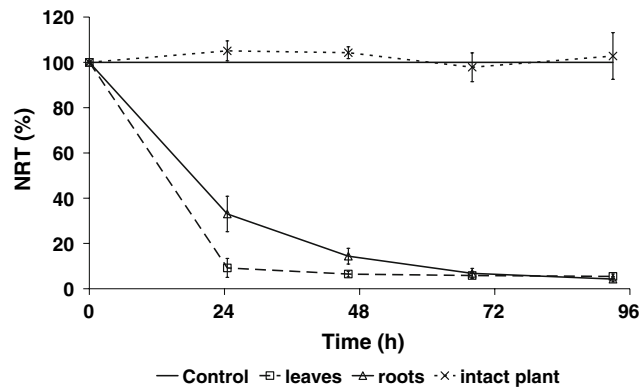


Fig. 3 NRT of willows grown in hydroponic solution containing 4-CP concentration of 7.4 mg/L. Roots and leaves refer to the experiments with detached roots and leaves, respectively. Error bars = SD

gibba) and *Selenastrum capricornutum*, which have been reported to have EC₅₀s of 23.5 and 29 mg/L, respectively (Sharma et al. 1997; Shigeoka et al. 1988). However, willow trees were at least as sensitive to 4-CP as green algae, *Chlorella vulgaris*, which was reported to have an EC₅₀ of 38 mg/L (Shigeoka et al. 1988). 4-CP also seemed to be more toxic to willows than to most of the other tested terrestrial plants, such as cabbage and millet (*Panicum milliaceum*). The EC₅₀ values for these species in root-elongation tests were reported to be 47.4 and 130 mg/L, respectively (Feng et al. 1996; Wang 1985).

Comparison of 4-CP, Phenol, and 2,4-DCP Toxicities to Willow Trees

The toxicity from phenol compared with monochlorophenols and dichlorophenols increased with the number of chloride atoms attached to the phenol ring. The respective approximate EC₅₀ values for phenol, 4-CP, and 2,4-DCP were 500, 32.2, and 10 mg/L based on the 3-day willow tree transpiration tests (Ucisik & Trapp 2006; Ucisik et al. 2007). The higher toxicity of the higher chlorinated

phenols might be explained by increased lipophilicity, which leads to a greater potential for uptake into the plant. Furthermore, the toxicity of substituted phenols was contributed to their uncoupling activity of electron transfer in membranes (Escher et al. 1999). The concentration ratio between biomembranes and external solution can be described partially by the partition coefficient between n-octanol and water, *i.e.*, K_{OW} (Trapp 2004). Therefore, a higher K_{OW} leads to a higher concentration in the membrane and subsequently to higher toxicity (*i.e.*, lower EC₅₀). The K_{OW} values for phenol, 4-CP, and 2,4-DCP were 30.2, 263, and 1,288 L/L⁻¹, respectively (Rippen 1991), and, in fact, the product of the EC₅₀ in the willow tree toxicity test and the K_{OW} for the three compounds was relatively constant (15,100, 8,469, and 12,882 for phenol, 4-CP, and 2,4-DCP, respectively) suggesting that the internal concentration in the membrane is the relevant concentration for the toxic effects observed in the test.

4-CP Loss and Accumulation

Two major mechanisms exist by which there is chemical loss from the system. The first mechanism is volatilization to air, either directly from the flask or after uptake into trees and translocation to leaves. The second mechanism is removal by metabolism, either by microorganisms or by the trees. A closer look at the results obtained in the experiments may help evaluate the relevance of these processes.

Loss from treeless flasks was on average 8%, which indicates some loss by volatilization. Accumulation in leaves was not observed (lower than detection limit) in any of the treatments with willows. This indicates that if there was a translocation of 4-CP to leaves, it was followed by volatilization or metabolism. Translocation of the chemical to leaves cannot be faster than the flow of the transport-medium water. For neutral organic compounds, the concentration ratio between transpiration stream and external solution, the so-called transpiration stream

concentration factor, is maximally 1 but usually lower (Briggs et al. 1982; Trapp 2000). Therefore, if 4-CP was taken up by trees together with transpiration water, the concentration in the remaining solution would not decrease by this process (but rather eventually increase); however, the mass of 4-CP would decrease because the volume of water also decreases. However, in all experiments, the final concentrations in the remaining solution decreased to values far lower than the initial concentrations (Fig. 2). In addition, when leaves were detached, loss from the system was as high as the loss observed for the intact plants (Table 4). Therefore, loss of chemical by direct volatilization and by translocation to leaves with subsequent volatilization can not be the dominating fate process. However, loss from the system was drastically decreased in the experiment using rootless trees. This provides evidence that the major reason for the loss of 4-CP from the media was degradation in the root zone, either by microbes located in or on roots or by root enzymes.

The accumulation of 4-CP differed among the individuals of willow cuttings in the roots exposed to the same 4-CP concentrations (replicates; Table 2). The high variation of the results was not surprising. In recent studies with phenol, 2,4-DCP, and cyanide, a nonlinear relation between uptake, metabolism, and toxicity was found, and small changes in the properties of the trees resulted in large changes in accumulation (Ucisik et al. 2007; Ucisik & Trapp 2006; Larsen et al. 2005).

Final 4-CP-concentrations in roots were similar for all exposure levels and, for intact trees, were all lower than the equilibrium concentration (*i.e.*, the RCF). A plausible explanation for this is that the loss of 4-CP occurred by way of metabolism by bacteria in the vicinity of the roots. Bacterial metabolism increases with increasing substrate availability (Monod kinetics). Therefore, the loss is faster at higher initial solution concentrations, leading to similar 4-CP levels in the roots at the end of the experiment despite different initial concentrations.

Rhizodegradation as a Treatment Option for 4-CP

Chlorophenols are frequently found in raw wastewater and sewage sludge. Concentrations of 4-CP in raw wastewater ranged from 200 ng/L to 2 mg/L (Rippen 1991). Concentrations in sewage sludge ranged from 0.028 to 90 mg/kg dry weight (mean 17) (Rippen 1991). The freely dissolved concentration in porewater can be estimated from the adsorption to organic carbon and is approximately half of that.

Subsequently, the concentrations of 4-CP in contaminated environments are usually lower than the EC_{10} (*i.e.*, 12 mg/L) for willows determined in this study, and the

occurrence of 4-CP does not inhibit the growth of willows. Therefore, root-zone degradation using willows is an option to treat wastewater or sludge that is contaminated with 4-CP, if concentrations are in the ranges given previously. It was shown in this study that 4-CP is rapidly degraded in the root zone and does not accumulate in stem or leaves at any nontoxic concentration. The amount volatilizing to air was also low. Therefore, phytoremediation is feasible without the risk of spreading the pollutant or contaminating the wildlife food chain.

Conclusion

The transpiration of willow trees (*S. viminalis*) was either not inhibited at all, or was only inhibited to a minor extent, by 4-CP levels ≤ 14.5 mg/L. The EC_{50} was 32.2 mg/L, whereas the respective EC_{50} s for phenol and 2,4-DCP were 500 and 10 mg/L, respectively. The trees did not survive at 4-CP concentrations ≥ 79.9 mg/L. Degradation in the root zone was the main reason for the loss of 4-CP. Phytoremediation of 4-CP using willow trees can be considered an efficient remediation option because of both its high removal efficiency and negligible accumulation inside plant tissue.

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