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Response of weeping willows to linear alkylbenzene sulfonate

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Abstract

Linear alkylbenzene sulfonate (LAS) is the most commonly used anionic surfactant in laundry detergents and cleaning agents. LAS compounds are found in surface waters and soils. The short-term acute toxicity of LAS to weeping willows (*Salix babylonica* L.) was investigated. Willow cuttings were grown in hydroponic solution spiked with LAS at 24.0 ± 1 °C for 192 h. The normalized relative transpiration of plants was used to determine toxicity. Severe reduction of the transpiration was only found for high doses of LAS ($\geqslant 240$ mg l^{-1}). Chlorophyll contents in leaves of treated plants varied with the dose of LAS, but there was no significant linear correlation. The activities of the enzymes superoxide dismutases (SOD), catalase (CAT), and peroxidase (POD) were quantified at the end of experiments. At higher concentrations of LAS ($\geqslant 240$ mg l^{-1}), the activities of SOD and CAT were decreased. The correlation between the dose of LAS and the POD activity in leaf cells was the highest of all enzyme assays ($R^2 = 0.5$). EC_{50} values for a 50% inhibition of the transpiration of the trees were estimated to 374 mg l^{-1} (72 h) and 166 mg l^{-1} (192 h). Results from this experiment indicated that phytotoxic effects of LAS on willow trees are not expected for normal environmental conditions.
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Keywords: LAS; Linear alkylbenzene sulfonate; Toxicity; Transpiration; Willows; Enzyme activity

1. Introduction

Linear alkylbenzene sulfonate (LAS), the most important group of synthetic anionic surfactant today, was introduced in 1964 as the readily biodegradable replacement for highly branched alkylbenzene sulphonates (Holmstrup and Krogh, 2001). The European chemical industry estimated a total annual consumption volume of 338 ktons. Most of LAS European consumption is in household detergents (>80%). The remainder of the LAS (<20%) is used in industrial and institutional cleaners, textile processing as wetting, dispersing and cleaning agents, industrial processes as emulsifiers, polymerization and in the formulation of crop protection agents (Carlsen et al., 2002). In mainland China, the annual production of synthetic anionic surfactants is about 1.0 million tons and more than 60% of the total is LAS (Liu et al., 2003).

The average use of LAS in Germany in the year 2000 was 386 g capita^{-1} = 1.1 g capita^{-1} d^{-1} (Wind et al., 2004), while the average use of household water between 1990 and 2001 was 127 l capita^{-1} d^{-1} (UBA, 2005). This yields an average concentration of LAS in household wastewater (before sewage treatment) of 8.6 mg l^{-1}, which is in agreement with the data (1–15 mg l^{-1} LAS in raw sewage) given by HERA (2004). During sewage treatment, most of the LAS is removed from the wastewater. Concentrations of LAS in rivers are in the microgram range, e.g. for the small German river Itter, values between 7 and 11 μg l^{-1} were measured (Wind et al., 2004). However, raw wastewater may be used as irrigation water, thus

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plants can be exposed to LAS in the milligram per liter range. Furthermore, wastewater may be treated in constructed wetlands or by willow stands. One objective of this study was therefore to determine the effect of LAS on exposed willows.

High concentrations of LAS were found in sewage sludge after anaerobic treatment. In Denmark, the highest observed LAS concentration in sludge was 16.1 g kg^{-1}. LAS could therefore be found in soils that were treated with sewage sludge as a fertilizer. With an application rate of 2 tons per hectar, the concentrations in the top 15 cm of soil would typically be from 0.1 to 1 mg kg^{-1}, with higher concentrations immediately after application of sewage sludge (Bro-Rasmussen and Solbé, 1999). In seven soil samples that were collected immediately after dosing of the fields with sludge, the concentration of LAS ranged from 2.5 to 40.3 mg kg^{-1} (median 25 mg kg^{-1}) (Painter, 1992). In sludge-amended soils, LAS had a half-life of about 1 week and monitored concentrations were around 1 mg kg^{-1} (max 1.4 mg kg^{-1}) at harvesting time (Painter, 1992). Sewage sludge application is therefore another likely pathway for the exposure of terrestrial plants to LAS.

Toxic effects of chemicals on plants can be measured in different ways. In plants, environmental adversity often leads to the increased generation of reduced oxygen species and, consequently, superoxide dismutases (SOD), catalase (CAT), and peroxidase (POD) have been proposed to be important in plant stress tolerance (Tsang et al., 1991). Adequate defense against oxygen toxicity requires efficient scavenging of both O^{2-} and of H_2O_2. Superoxide radicals (O^{2-}) are toxic by-products of oxidative metabolism (Fridovich, 1978). Their toxicity has been attributed to their interaction with hydrogen peroxide to form highly reactive hydroxyl radicals (OH^-), which are thought to be largely responsible for mediating oxygen toxicity in vivo. SOD are metalloproteins that catalyze the dismutation of superoxide radicals (O^{2-}). CAT and POD catalytically scavenge H_2O_2 and provide the necessary defenses (Fridovich, 1989). Furthermore, a decrease in chlorophyll content in green plants in response to environmental stress has been reported (Fodor, 2002). Other effects may be reduced growth and transpiration, or changes in water use efficiency. Trapp et al. (2000) specially designed a rapid acute phytotoxicity test for chemicals in water or in soil using the transpiration of basket willow (*Salix viminalis*) cuttings. Instead of the European basket willow, the Chinese weeping willow (*Salix babylonica* L.) was used in this study, which can be handled similar to basket willows.

This study investigates the response of the native Chinese species *Salix babylonica* L. exposed to LAS, with the objectives to compare the toxic effects and to provide quantitative information whether the toxicity of LAS would allow wastewater treatment, sewage sludge application in willow stands, or phytoremediation of LAS with willows.

2. Materials and methods

2.1. Trees and exposure regimes

Weeping willow (*Salix babylonica* L.) was sampled from nature at the campus of the Hunan Agricultural University, China. Forty centimeter long tree cuttings were removed from mature specimens and all from a single tree. They were placed in buckets of tap water at room temperature of 15–20 °C under natural sunlight until new roots and leaves appeared. After a 2-month growth, pre-rooted cuttings were transferred to 250 ml Erlenmeyer flasks filled with approximately 200 ml modified ISO 8692 nutrient solution (Table 1). The flasks were all sealed with cork stoppers and play dough to prevent escape of water or chemicals, and wrapped with aluminum foil to inhibit algae growth. For each treatment concentration, six replicates were measured. The flasks were put in a climate chamber with a constant temperature of 24.0 ± 1 °C under continuous artificial light. The plants remained there 48 h to allow them to adapt to their new living environment. Then, the weight of the plant system was measured. Twenty-four hours later, the flasks with the trees were weighed again. By this, the transpiration was determined. Trees with similar transpiration were selected for the tests. The nutrient solution of these trees was exchanged to spiked solution, except for controls. Dodecyl-benzene sulfonic acid sodium salt ($CH_3(CH_2)_{11}C_6H_4SO_3Na$) was used in this study. The LAS used was p.a. grade (*per analysis*, in China: ⩾95% purity). It had a chain length of C_{12} and the benzene ring was attached to C_6. Six different treatment concentrations of LAS were applied (0, 30, 60, 120, 240, 360 and 480 mg l^{-1}). The weight of the flasks was measured daily.

2.2. Normalized relative transpiration

The weight loss, compared to initial loss, was the toxicity criteria. To compare the toxic effect on cuttings with different initial transpiration (before the toxicant is added), the weight loss is expressed as relative transpiration. The transpiration was normalized with respect to the initial transpiration (to eliminate the necessity of finding cuttings with similar initial transpiration) and with respect to the transpiration of uncontaminated control cuttings (to include the effect of normal growth of the cuttings during the test). The mean normalized relative transpiration (NRT) was calculated by

Table 1
Composition of the modified ISO 8692 nutrient solution

Macronutrients (μmol l^{-1})		Micronutrients (nmol l^{-1})	
$NaNO_3$	2823.9	HBO_3	2992.1
$MgCl_2 \cdot 6H_2O$	59.0	$MnCl_2 \cdot 4H_2O$	2097.0
$CaCl_2 \cdot 2H_2O$	122.4	$ZnCl_2$	22.0
$MgSO_4 \cdot 7H_2O$	60.9	$CoCl_2 \cdot 6H_2O$	6.3
KH_2PO_4	246.0	$CuCl_2 \cdot 2H_2O$	0.1
$MaHCO_3$	1785.5	$NaMoO_4 \cdot 2H_2O$	28.9

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