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Alleviation of Cd toxicity by composted sewage sludge in Cd-treated Schmidt birch (*Betula schmidtii*) seedlings

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Abstract

We investigated alleviation of Cd toxicity and changes in the physiological characteristics of *Betula schmidtii* seedlings following application of composted sewage sludge to Cd-treated plants. Plants were grown under four test conditions: control, Cd treatment, sludge amendment, and Cd treatment with sludge amendment. *B. schmidtii* treated with Cd only accumulated the greatest amount of Cd in the leaves, but absorbed Cd was also highly concentrated in the roots. In contrast, Cd concentrations in the Cd and sludge amendment treated seedlings were the lowest in the roots. Since sludge amendment increased the growth of seedlings, it may have alleviated toxicity by dilution of Cd. Additionally, the absorbed Cd was more widely distributed since it was transported from the roots and accumulated in the stems and leaves of Cd and sludge treated plants. Cd treatment inhibited the growth and physiological functions of *B. schmidtii* seedlings, but sludge amendment compensated for these effects and improved growth and physiological functions in both Cd-treated and control plants. SOD activity in the leaves of seedlings was increased in the Cd-treated plants, but not in the Cd and sludge amendment treated seedlings. In conclusion, alleviation of Cd toxicity in response to sludge amendment may be related to a dilution effect, in which the Cd concentration in the tissues was effectively lowered by the improved growth performance of the seedlings.

Keywords: Sludge amendment; Physiological functions; SOD activity; Dilution effect; Growth performance

1. Introduction

Soil pollution by heavy metals has become a critical environmental concern due to its potentially adverse ecological effects. Heavy metals occur naturally at low concentration in soils. However, they are considered soil contaminants due to their widespread occurrence, acute and chronic toxicity. Among the heavy metals, cadmium (Cd) is of special concern due to its relatively high mobility in soils and potential toxicity to biota at low concentrations (Das et al., 1997). Cd is a nonessential heavy metal that does not have any metabolic use. Cd is naturally present in soils in trace amounts, but high levels of Cd have been reported in some soil environments. Jung and Thornton (1996) reported up to 40 mg kg^{-1} Cd concentrations in surface soils taken from a mining area in Korea.

Recently many researchers have tried to find suitable materials and technology for remediation of metal-contaminated soils. As a result, a number of plants which have an ability to accumulate specific heavy metals have been identified, and the biochemical mechanisms for accumulation and defense against heavy metals have been investigated (Choi et al., 2001; Milone et al., 2003; An, 2004; Tong et al., 2004). Plants have developed a variety of mechanisms to tolerate heavy metals, which can severely damage plants by inhibition of metabolic pathways (Salt et al., 1998; Toppi and Gabbrielli, 1999). For example, one of the major defense mechanisms in plants is to inactivate

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metal ions by chelation with compounds such as metallothioneins and phytochelatins (Steffens, 1990; Zenk, 1996).

However, these results are difficult to apply to real field sites since most experiments are lab based, which are quite different from natural environmental conditions. Therefore, to overcome these limitations, we have selected pioneer tree species which are naturally able to colonize polluted soils and have evaluated their tolerance and accumulation of heavy metals (Lee et al., 2002, 2003; Oh et al., 2004a,b). Further work is aimed at identifying and improving nutrient deficiencies that interfere with growth performance in contaminated soils like mine tailings (Hossner and Hons, 1992; Pichtel and Salt, 1998).

We selected *Betula schmidtii* as a suitable candidate for revegetation of tailings and developed a composted sludge as a soil amendment to compensate for nutrient deficient conditions (Lee et al., 2002, 2003). In addition, we suggested that the sludge amendment could improve the physiological and biochemical properties of *B. schmidtii* grown on nutrient deficient, toxic metal containing mine tailings (Han et al., 2004).

Therefore, this study was conducted to investigate beneficial or deleterious effects of the composted sludge amendment on Cd accumulation, and on the physiological and biochemical properties of *B. schmidtii* seedlings treated with Cd.

2. Materials and methods

2.1. Plant materials and treatments

Schmidt birch (*B. schmidtii*) was selected from the pioneer tree species found on the abandoned coal-mine lands located at Sododong in the Taebaek region of Korea (Lee et al., 2002). We collected seeds of this species from a wild population growing in the mine tailings on this site. The seeds were planted in containers with vermiculite in early spring 2003.

At seven weeks after planting, 20 seedlings (five replicates each for four treatments) of the same height (≈10 cm) were transplanted to individual plastic pots (15 cm D × 20 cm H). The four treatment groups were: untreated controls, Cd treatment, sludge amendment treatment, and both Cd and sludge amendment treatment. For the Cd treatment, seedlings were given 200 ml of 600 μM $CdSO_4$ solution every 3 d, and the pots were placed in plastic dishes to retain leached nutrients and the $CdSO_4$ solution. Sludge amendment consisted of 5 g of the composted sludge soil per pot. The composted sludge was made from sewage sludge and several additives (sawdust and microbial additives), which had been allowed to ferment for approximately 45 d; the sludge amendment was pH 6.05, 45.8% organic content, C/N ratio of 15.1 and a total nitrogen content of 2.52%.

Pots were randomized in the greenhouse, watered from above daily, and moved about every two or three weeks throughout the 45 d experimental period to minimize positional effects. During the experimental period, daily mean temperature and relative humidity were 23.1 ± 2.1 °C and 74.3 ± 10.9%, respectively.

2.2. Measurements and statistical analysis

At the end of the 45 d trial, the relative growth rate (RGR), as indicated by height, was calculated as: $RGR = [\ln(X_2) - \ln(X_1)]/(t_2 - t_1)$, where X_1 was the height at time t_1 (start of the experiment) and X_2 was the height at time t_2 (termination of the experiment). Leaf chlorophyll content was determined with a chlorophyll meter (SPAD-502, Minolta, Osaka, Japan).

Photosynthetic properties were measured with a portable photosynthesis system (LI-6400, LI-COR Biosciences, Inc., Lincoln, NE, USA) at the conclusion of the 45 d experimental period.

Three fully expanded stem-attached leaves (leaf position from fourth to sixth from the top) per plant were measured at light saturation (1200 $\mu mol\ m^{-2}\ s^{-1}$), which was determined from a light-response curve (between 0 and 2000 $\mu mol\ m^{-2}\ s^{-1}$), provided by an LED light module, and the steady-state rate of photosynthesis was calculated. The CO_2 concentration during measurements was maintained between 340 and 360 μmol CO_2 mol^{-1} air, leaf temperature was 24.0 ± 0.2 °C and RH was 60 ± 5%. Data from two replicate measurements were averaged for each plant. A leaf CO_2 uptake (A) versus intercellular CO_2 concentration (C_i) curve, A/C_i curve, was determined using a range of ambient CO_2 concentrations (between 0 and 350 μmol CO_2 mol^{-1} air), and carboxylation efficiency was then estimated based on the method of Farquhar et al. (1980).

Leaves of the freshly harvested plants were used for the determination of protein content and superoxide dismutase (SOD) activity. Protein content in the leaves of the control and treated plants was determined by the method of Lowry, using bovine serum albumin as a standard protein (Lowry et al., 1951). SOD activity assay per 100 mg protein was measured using the nitro blue tetrazolium-xanthine oxidase method (Beauchamp and Fridovichi, 1971).

At harvest, shoots and roots were carefully removed, and then thoroughly rinsed with distilled water twice. After oven drying of the tissues at 70 °C to constant weight, the dry-weight yields of shoots and roots were recorded. Dried shoots and roots (0.5 g each) were ground and used to determine Cd concentration. Nitric acid (70%, 15 ml) and hydrogen peroxide (30%, 5 ml) were added to 0.5 g of dried, ground plant sample in a digestion vessel. Samples were digested using the microwave digestion system, cooled after addition of distilled water, and filtered prior to analysis. Cd content in the digested tissue was measured by atomic absorption spectrophotometer (AA-6701 F, Shimadzu, Tokyo, Japan).

The data were statistically analyzed using SAS for Windows Version 8.1 (SAS Institute Inc., Cary, NC, USA). Mean values per treatment were compared by ANOVA.