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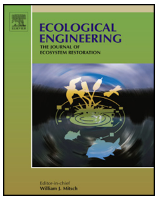

Sewage sludge fertilization in larch seedlings: Effects on trace metal accumulation and growth performance

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ABSTRACT

The spreading of sewage sludge (SS) among forest plantations may provide interesting results for firewood production. While sludges are good fertilizers, they may nevertheless contain trace metals, which can reduce productivity and lead to environmental risks. We investigated the effects of SS application on nutrient uptake and growth parameters in larch seedlings (*Larix decidua*) and determined trace metal and mineral distribution. Without incorporation into the soil, sludge was applied to the soil surface at three rates (0, 30 and 60 t dry weight DW ha^{-1}). The plants were harvested after 12 months. The results showed significantly increased nitrogen and phosphorus concentrations in the top soil layer in pots amended with sludge, whereas no changes appeared in the lower layers. Similar results were obtained for the Cu, Zn and Cd concentrations. However, no differences were observed for the other measured soil mineral elements. Nitrogen concentrations in needles increased with rising sewage sludge application rates, yet the sludge had no effect on the P, Mg, Zn, Pb and Cd concentrations. In addition, Cu accumulated only in the lateral roots of seedlings that received the highest sludge loading rate. Sludge application improved the net photosynthesis, which resulted in higher chlorophyll contents in the needles. Following application, the dry matter accumulation rate increased due to the excessive availability of N, whereas available mineral elements in the plant tissues were diluted. Furthermore, amending the soil with sewage sludge can promote a higher biomass yield which may result in an increased trace metal bioaccumulation capacity in plants. Though this investigation has established the benefits of municipal SS application, further studies are needed to assess the potential transfer of TM to groundwater and through the food chain.

1. Introduction

Wastewater plant operators will continue to face the challenge of disposing of millions of tons of sewage sludge (SS) generated each year (Japan: 70 million, China: 30 million, USA: 6 million and France: 1.2 million) (Matsubara and Itoh, 2006; McClellan and Halden, 2010; Kelessidis and Stasinakis, 2012; Legroux and Truchot, 2009). As SS has a high nutrient content, its increased use as a fertilizer has been recommended as the best practical environmental option for the management of this organic residue. In France about 73% of sludge produced is applied to agricultural land (Legroux and Truchot, 2009). However, sludge contains several potentially harmful constituents such as trace metals (TMs) and organic pollutants which may accumulate in agricultural soil over time (Lopez-Mosquera et al., 2000; McBride, 2003; Chopra et al., 2009). Once accumulated, TMs are highly persistent in the topsoil, can cause potential problems or be transferred through the food chain (Alloway et al., 1991), thus posing a threat to human health (Wang et al., 2003; Chopra et al., 2009). In the case of forest plantations receiving SS application, the risks are mitigated since farmed trees are not a direct part of the human food chain. In addition, this alternative application is promoted as it is thought to enhance both tree growth and wood production (Henry and Cole, 1997; Mosquera-Losada et al., 2001; Bramryd, 2001; Tsakou et al., 2003; Vaitkuté et al., 2010) and to improve several soil characteristics (Barzegar et al., 2002; Veeresh et al., 2003; Hussein, 2009). In France, application of SS to forest land is currently in the experimental phase and requires intensive monitoring of

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ecosystem components such as plant, soil, water and fauna, to identify any potential positive and/or negative effects on plant productivity and environmental integrity (Bourioug et al., 2015).

The objectives of this investigation were to study the balance between the beneficial effects and eventual toxicity of SS applications on young larch seedlings (*Larix decidua*) by measuring the effects of two different sludge doses (30 and 60 t dry matter ha^{-1}). Several growth parameters, biomass productivity and nitrogen, as well as phosphorus were examined. TM uptake and transfer within plant tissues were also investigated.

2. Materials and methods

2.1. Origin and characterization of soil and sludge

The soil used in this experiment was a pseudo luvisol with dysmull, collected from a forested area located at Mélisey, Haute-Saône, France (47°753′ latitude, 6°580′ longitude). Soil samples were collected from within the top 20 cm of the soil layer and sieved through a 1 cm mesh. Aerobically digested SS from a domestic wastewater treatment plant in the village of Mélisey was also used. The physico-chemical characteristics of the soil and SS are provided in Table 1.

2.2. Plant material, growth conditions and experimental setup

One-year-old *L. decidua* (European larch) seedlings were purchased from a local nursery. On December 22, 2008 the soil samples were placed in 7.5 l plastic pots, each one filled with 6.7 kg of the sampled soil (Fig. 1). During the first 30 days the pots were watered with distilled water three times per week to maintain a constant weight (70% of field capacity). SS was then applied to the soil surface without incorporation into the soil. The rate of sludge was equivalent to (30 t dry weight (DW) ha^{-1}: 30S) or twice (60 t DW ha^{-1}: 60S) the maximum accumulated amount permitted under French law (i.e., 30 t DW ha^{-1} over a 10-year period). Pots without sludge (0S) were used as the control. Each configuration was replicated five times. The plants were grown for 12 months in a greenhouse with a 14 h photoperiod (200 μmol m^{-2} s^{-1}), a temperature regime of 25/14 °C (day/night) and 60% relative humidity.

2.3. Photosynthesis measurement and chlorophyll content

The CO_2 assimilation rate (Pn), stomatal conductance (Gs) and intercellular CO_2 concentration (Ci) were measured using a Li-COR6400 portable photosynthesis system (LI-COR-6400, LI-COR Biosciences, Inc., Lincoln, Nebraska, USA) connected to a conifer chamber. Monthly measurements were obtained from June to September under the ambient conditions of the culture chamber (i.e., 25 °C, 60% RH and 200 μmol m^{-2} s^{-1}). Only the CO_2 concentration was maintained at 400 μmol s^{-1}. In addition, needles were collected from both sides of the enclosed branches that were used for gas exchange measurements; the projected areas of the needles were digitally determined in the laboratory. After at least 5 min of steady readings in the chamber, the photosynthesis rate was recorded. All measurements were obtained from a single branch at the same distance from the stem apex. Data were recalculated according to the measured needle area. The collected needles were used to quantify the chlorophyll content, which was extracted using 100% dimethyl sulfamide (Robakowski et al., 2004) and was calorimetrically determined according to Barnes et al. (1992). The results were expressed as milligram of chlorophyll per gram of fresh matter (FM) (mg g^{-1}).

2.4. Sampling

At harvest, an aerial part of each plant was removed, which, by means of the Scholander chamber method, permitted immediate determination of water potential. The aerial part was then cut into separate organs, i.e., needles, the main stem and ramifications. Afterward, the root portion was carefully removed from the pot, separated from the adhering soil and washed with distilled water. The lateral roots were removed from the taproot after their lengths had been measured. Each sample was lyophilized. Also, the dry mass of each sample was determined before being reduced to a fine powder using a ball mill (Retsch MM 200, Germany). Soil samples were simultaneously collected from three levels in each pot as follows:

(i) the sludge layer (Sl), corresponding to the upper layer of the pot with a depth of approximately 2 cm. This layer is primarily composed of organic matter that was previously contained in the sludge and formed via mineralization;

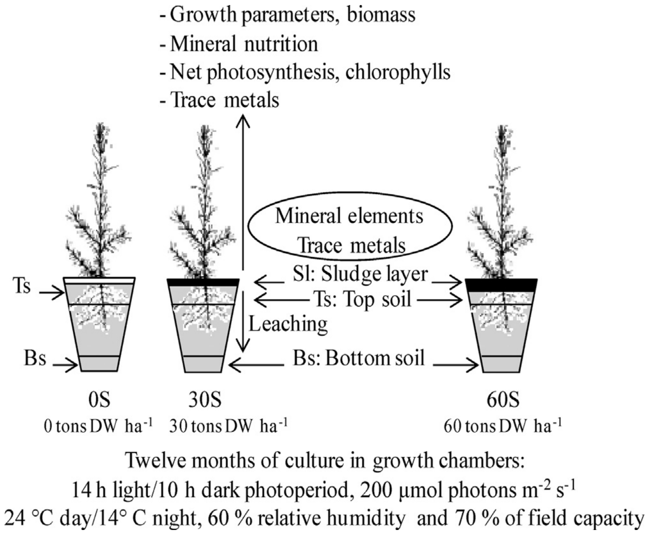

Fig. 1. Schematic representation of the experimental design.