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Research article

Responses of Szarvasi-1 energy grass to sewage sludge treatments in hydroponics

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ABSTRACT

Sewage sludge (SS) originating from communal wastewater is a hazardous material but have a potentially great nutritive value. Its disposal after treatment in agricultural lands can be a very economical and safe way of utilization once fast growing, high biomass, perennial plants of renewable energy production are cultivated. Szarvasi-1 energy grass (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1), a good candidate for this application, was grown in hydroponics in order to assess its metal accumulation and tolerance under increasing SS amendments. The applied SS had a composition characteristic to SS from communal wastes and did not contain any toxic heavy metal contamination from industrial sludge in high concentration. Toxic effects was assessed in quarter strength Hoagland nutrient solution and only the two highest doses (12.5–18.75 g dm^{-3}) caused decreases in root growth, shoot water content and length and stomatal conductance whereas shoot growth, root water content, chlorophyll concentration and the maximal quantum efficiency of photosystem II was unaffected. Shoot K, Ca, Mg, Mn, Zn and Cu content decreased but Na and Ni increased in the shoot compared to the unamended control. The nutritive effect was tested in 1/40 strength Hoagland solution and only the highest dose (12.5 g dm^{-3}) decreased root growth and stomatal conductance significantly while lower doses (1.25–6.25 g dm^{-3}) had a stimulative effect. Shoot K, Na, Fe and Ni increased and Ca, Mg, Mn, Zn and Cu decreased in this treatment. It was concluded that SS with low heavy metal content can be a potentially good fertilizer for high biomass non-food crops such as Szarvasi-1 energy grass.

1. Introduction

Sewage sludge (SS) from a communal wastewater-treatment plant is a toxic and biologically hazardous product, which requires careful handling before deposition. With the development of industrialization and urbanization, large amount of sewage is generated. Thus, the management of SS is an issue of growing importance. It usually contains pathogenic microorganisms as well as organic constituents and heavy metals in different composition and concentration. Therefore, in all countries of the European Union, there are directives for storage, stabilization and safe recycling. Before recycling, SS treatment usually involves disinfection, digestion, composting, and heat drying (Epstein, 2003; Fytili and Zabaniotou, 2008; Yang et al., 2015; Cieślik et al., 2015). During the disinfection process, the microflora of SS is changed. However, these treatments generally do not modify the heavy metal content. Storage, deposition or utilization of the SS depends on the concentration and bioavailability of heavy metals (Uri and Simon, 2008). Nevertheless, dilution and composting makes it possible to utilize its fertilizer potential due to its high N, P and K content (Epstein, 2003). The application of SS compost on degraded lands rated not suitable for agricultural food production may indeed lead to land utilization in renewable energy production.

Fast growing, large biomass perennial plants, such as Szarvasi-1 energy grass (*Elymus elongatus* subsp. *ponticus* cv. Szarvasi-1) (Csete et al., 2011; Martynak et al., 2017) require increasing nutrient resupply to the soil which can be provided by SS compost.

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The SS is a good source of plant nutrients such as N, P, K, Ca or Mg (Singh and Agrawal, 2010). Heavy metals such as Cu and Zn are often abundant in SS but these are also micronutrients for plants. Whereas non-essential ones, like Cd and Pb are often toxic and may have deleterious effect on plants (Marschner, 1995). In order to avoid decreased yield it is important to test potential plant varieties for sensitivity or tolerance to the heavy metals. Szarvasi-1 energy grass has recently been tested for tolerance to Cd, Cu, Ni, Pb and Zn in nutrient solution and it proved to be tolerant to Ni and Pb while sensitive to Cd and Cu. Furthermore, this plant not only accumulates Zn but its growth is also stimulated by 10 μM $ZnSO_4$ in the nutrient solution (Sipos et al., 2013).

There is plenty of information on the nutritive effect of the SS (Singh and Agrawal, 2010). Nevertheless, overdosed deposition may lead to the induction of stress symptoms due to high nutrient or heavy metal content (Smith, 2009). In the present study we tested the Szarvasi-1 energy grass with exposure to communal SS in nutrient solution in order to assess the potential toxic effect of such treatment by applying increasing doses of the SS over the normal element composition of the nutrient solution and, on the other hand, to assess the fertilizer effect of the SS sample using diluted nutrient solution in a similar experiment.

2. Methods

2.1. Plant material and treatments

Seeds of the tall wheatgrass cultivar Szarvasi-1 energy grass (*E. elongatus* subsp. *ponticus* [Podp.] Melderis cv. Szarvasi-1, syn. *Agropyron elongatum*, *Elytrigia elongata*) (Csete et al., 2011) were germinated for seven days on wet filter papers in Petri dishes at room temperature and sunlight. Three seedlings with 2–5 cm long roots were placed on a 2 cm wide strip of sponge-rubber, rolled up and fastened in a polystyrene ring. The seedlings were transferred to plastic containers filled up with 10 dm^3 modified, continuously aerated, unbuffered, 1/4 strength Hoagland nutrient solution (H4) of the following composition: 1.25 mM KNO_3; 1.25 mM $Ca(NO_3)_2$; 0.5 mM $MgSO_4$; 0.25 mM KH_2PO_4; 11.6 μM H_3BO_3; 4.5 μM $MnCl_2 \cdot 4H_2O$; 0.19 μM $ZnSO_4 \cdot 7H_2O$; 0.12 μM $Na_2MoO_4 \cdot 2H_2O$; 0.08 μM $CuSO_4 \cdot 5H_2O$ and 10 μM Fe(III)–citrate-hydrate. The plants were grown in a climate controlled growth chamber at 20/25 °C, 75% relative humidity and 150 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) with 10/14 h dark/light period. The nutrient solution was continuously aerated and replaced with fresh solution once a week.

After 30 days of growth the plants were transferred to individual pots containing 0.8 dm^3 aerated nutrient solution in two treatment groups. One group was supplied with the same nutrient solution as during the pregrowth period (H4) but supplied also with 0, 1, 5, 10, 15 g dried SS directly added to the solution in each pot and left unfiltered throughout the treatment period. The other group was supplied with diluted nutrient solution of 1/40 strength (H40) and with 0, 1, 5, 10 g dried SS similarly as the other group. The SS was derived from a pilot-scale microaerophilic, thermophilic digester, where the temperature was 63 °C. The SS used in this experiment had an original dry matter content of 6.8%. The organic matter content was 6.5–7% on a dry matter basis. Element composition of the dried SS, deionized water and nutrient solution amended with SS and filtered to remove solid particles is shown in Table 1. Each pot was supplemented with 0.1 dm^3 deionized water after 7 and 14 days of the treatment period in order to refill the transpired/evaporated water and to avoid further supply of nutrients. In one experiment 3 parallel pots was used for the same treatment and the experiment was done in triplicate. At the end of the 3-week treatment period the plants were harvested for mass measurements and element analysis.

2.2. Mass measurements

The roots were thoroughly cleaned in deionized water to remove SS particles, then centrifuged between filter papers at 300 g and weighed to determine fresh mass. Dry mass of all tissues (shoots and roots separately) was determined after drying at 80 °C.

2.3. Chlorophyll concentration

The measurements were made after 8 days of treatment with the first fully developed leaves. The chlorophyll (Chl) concentration was determined spectrophotometrically (Shimadzu UV-2101PC) from 80% acetone extracts using the equations of Porra et al. (1989). Each measurement was carried out on three individual plants in each treatment group.

2.4. Stomatal conductance

Stomatal conductance was measured with a porometer (DELTA-T Devices Ltd.) on the adaxial epidermis of the middle sections of the youngest, fully developed leaves after 8 days of treatment. Transpiration was calculated as mmol H_2O m^{-2} s^{-1}. Each measurement was carried out three times on three individual plants in each treatment group.

2.5. Chlorophyll a fluorescence induction

Fluorescence induction measurements were carried out with intact leaves using a PAM 101-102-103 Chlorophyll Fluorometer (Walz, Effeltrich, Germany). Leaves were dark-adapted for 15 min. The F_0 level of fluorescence was determined by switching on the measuring light (modulation frequency of 1.6 kHz and PPFD less than 1 μmol m^{-2} s^{-1}) after 3 s illumination with far-red light in order to eliminate reduced electron carriers (Belkhodja et al., 1998). The maximum fluorescence yield of the dark-adapted stage, F_m was measured by applying a 0.7 s pulse of white light (PPFD of 3500 μmol m^{-2} s^{-1}, light source: KL 1500 electronic, Schott, Mainz, Germany). The maximal quantum efficiency of photosystem (PS) II centres were determined as $F_v/F_m = (F_m - F_0)/F_m$.

2.6. Element concentrations

Element content of the SS, the SS-containing media (amended deionized water and nutrient solution) and the shoots have been measured. Roots have not been measured as it was not possible to fully remove the SS material. Measurement of solution samples were made after filtration through MN 640 W filter paper. Measurements of the SS and plant samples were made after acidic digestion. 5–10 ml cc. HNO_3 was added to each gram of the samples for overnight incubation. Then the samples were pre-digested for 30 min at 60 °C. Finally, 2–3 ml H_2O_2 (30 m/m %) was added for a 90 min boiling at 120 °C. The solutions were filled up to 10–50 ml, homogenised and filtered through MN 640 W filter paper. The element content of the filtrate was determined by ICP-MS. All samples were prepared in triplicate.

2.7. Statistics

Basic statistical analysis was carried out with one-way ANOVA and Tukey-Kramer multiple comparisons *post-hoc* test ($P < 0.05$) using InStat 3.0 (GraphPad) software.