Szarvasi-1 energy grass efficiently utilizes high doses of sewage sludge in hydroponics

Ambrus Rév[a], Brigitta Tóth[b], Ádám Solti[c], Gyula Sipos[d], Ferenc Fodor[c,*]

[a]Department of Ecology, Faculty of Science and Technology, University of Debrecen, 1 Egyetem Sq., H-4010 Debrecen, Hungary.
[b]Department of Botany, Crop Physiology and Biotechnology, Institute of Plant Sciences, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, 138 Böszörményi Str., H-4032 Debrecen, Hungary.
[c]Department of Plant Physiology and Molecular Plant Biology, ELTE Eötvös Loránd University, Budapest, 1/C Pázmány P. lane, Budapest, H-1117, Hungary
[d]Agricultural Research and Development Institute, 30 Szabadság Str., H-5540, Szarvas, Hungary.

[*]corresponding author

E-mail:
Ambrus Rév, revambrus@gmail.com
Brigitta Tóth, btoth@agr.unideb.hu
Ádám Solti, adam.solti@ttk.elte.hu
Gyula Sipos, gyula.sipos@sonic.co.hu
Ferenc Fodor, ferenc.fodor@ttk.elte.hu

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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.

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 ssp. 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. The SS is a good source of plant nutrients such as N, P, K, Ca or Mg (Martinez et al. 2003). 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).

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.

Materials and Methods

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 elongate*) (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 $MnCl_2·4H_2O$; 0.19 μM $ZnSO_4·7H_2O$; 0.12 μM $Na_2MoO_4·2H_2O$; 0.08 μM $CuSO_4·5H_2O$ 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 original 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 (data were kindly provided by the company). 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.

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.

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.

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.

Fluorescence induction of chlorophyll a
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 yields, F_m in the dark-adapted state 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 $F_v/F_m = (F_m - F_0)/F_m$.

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.

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.

Results

Analysis of the SS

The SS applied in this experiment has been analysed as a dried material and as a dissolved additive in deionized water and H4 (10 g dm^{-3} dried SS in water or nutrient solution) (Table 1). The dried SS is rich in K, Na and Fe but otherwise contains low concentration of heavy metals such as Cu, Zn, Cd, Co, Pb. Deionized water dissolves nutrients and nonessential elements from the dried SS material. The elemental composition of the resulting solution is characterised by concentrations exceeding that of unamended, H4 for most macro and micronutrients measured except for Ca and Mn which are lower and Na which is much higher. Some nonessential elements (Al, Cr, Li, Sr, Ti) were also detected in the solution in low concentration. The H4 amended with SS contains all the measured elements in concentrations elevated in a variable extent as compared to the original composition except for Mn which was slightly lower.

Table 1. Element composition of the dried SS, deionized water and H4 containing 10 g dm^{-3} dried SS. For reference, the theoretical composition of the H4 used for control plants is also shown. (n=3, SD<5%, DL = detection limit.)

Element	dried SS	deionized water + dried SS	H4 + dried SS	H4 control
	mg kg^{-1}	μmol dm^{-3}	μmol dm^{-3}	μmol dm^{-3}
Ca	19468	770	1775	1250
K	6941	2414	4156	1500
Mg	3662	685	1345	500
P	15861	1075	1269	250
S	11788	850	1812	500
B	36	13.51	25.81	11.56
Cu	276	0.31	0.40	0.08
Fe	12896	11.55	15.60	10.00
Mn	164	0.56	4.06	4.60
Na	1367	797.83	813.04	0.24
Zn	389	0.56	0.84	0.19
Al	2270.00	2.68	3.76	0
Cd	4.95	< DL	< DL	0
Co	3.31	< DL	< DL	0
Cr	29.40	0.35	0.04	0
Li	1.36	0.91	0.91	0
Ni	11.00	< DL	< DL	0
Pb	11.30	< DL	< DL	0
Sr	216.00	1.36	1.52	0
Ti	50.80	0.03	0.02	0

Growth parameters

The root dry mass of control plants in H4 was almost half of that in H40 whereas the shoot dry mass was the same. The growth of plants has only been inhibited at the highest SS doses. Root dry weight was significantly decreased by 10 and 15 g dried SS while shoot dry weight was significantly changed only at the 15 g SS dose (Fig. 1).

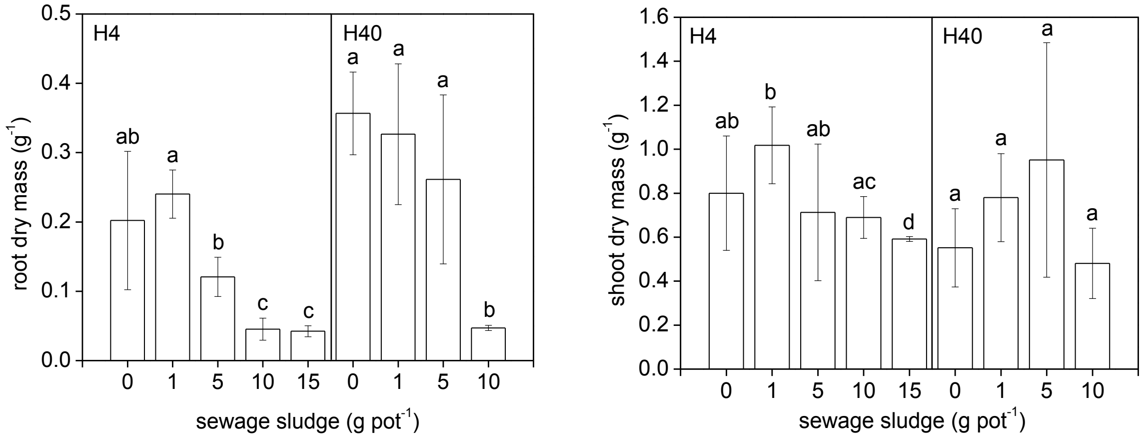

Figure 1. Root and shoot dry mass of Szarvasi-1 energy grass grown in H4 and H40 amended with 0, 1, 5, 10 and 15 g dried SS applied in 0.8 dm^3 pots. (mean±SD, n=3; values marked with different letters are significantly different at P<0.05)

The water content of both roots and shoots was markedly lower in H40 compared to H4 control plants. Root water content decreased only at the highest SS dose in H4 while in H40 it continuously increased with the increasing SS dose whereas shoot water content continuously decreased with the increasing SS doses in H4 but in H40 it continuously increased until the application of 5 g dried SS material (Fig. 2).

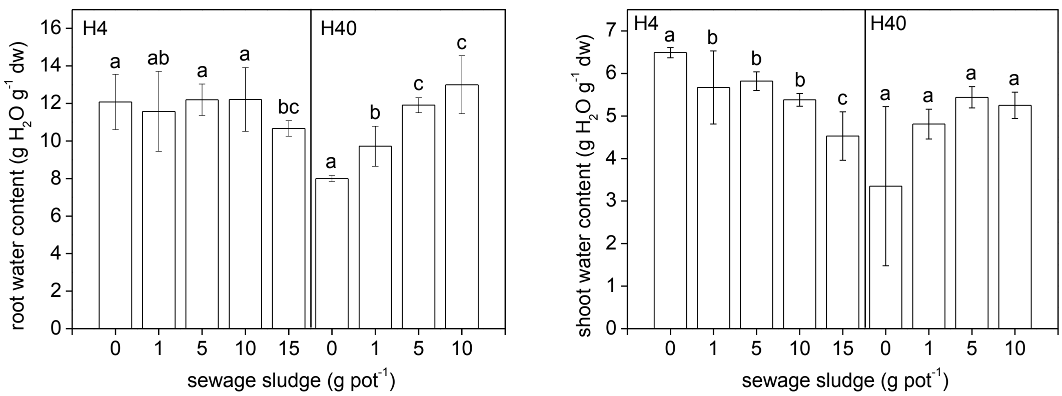

Figure 2. Root and shoot water content of Szarvasi-1 energy grass grown in H4 and H40 amended with 0, 1, 5, 10 and 15 g dried SS applied in 0.8 dm^3 pots. (dw=dry weight; mean±SD, n=3; values marked with different letters are significantly different at P<0.05)

Shoot growth was lower compared to the control at 5, 10 and 15 g SS amendments in H4 but 1 and 5 g treatments resulted in increased shoot length in H40 (Fig. 3).

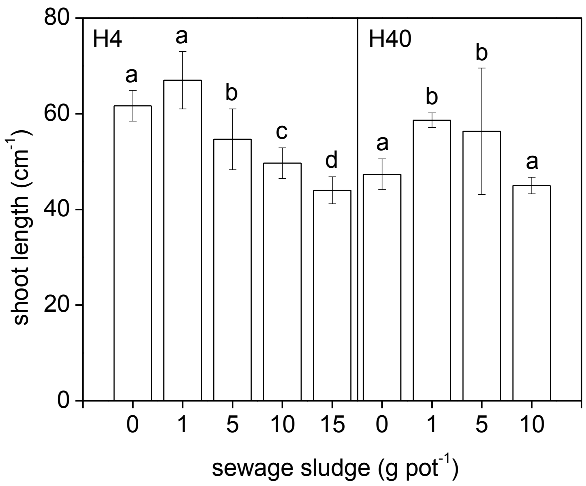

Figure 3. Shoot length of Szarvasi-1 energy grass grown in H4 and H40 amended with 0, 1, 5, 10 and 15 g dried SS applied in 0.8 dm^3 pots. (mean±SD, n=3; values marked with different letters are significantly different at P<0.05)

Physiological parameters

The pH of the nutrient solution during the treatment period has increased in all cases as compared to the initial value in the fresh control and amended solutions (Fig. 4). The highest pH (8.5) was detected in H4 at 10 and 15 g SS doses while the lowest one (6.9) was in H40 at 1 g dose. The highest increase of almost 3 pH units was observed in the control H4.

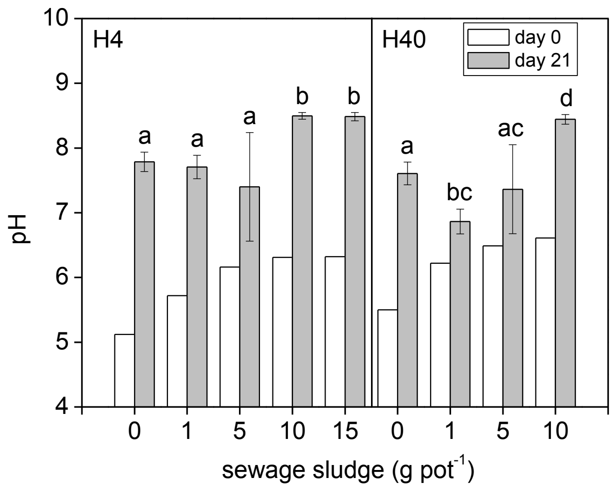

Figure 4. pH of the nutrient solution (H4 and H40) at the time of treatment with different doses (0, 1, 5, 10 and 15 g dw in 0.8 dm^3 pots) of SS (day 0) and at harvest (day 21). The nutrient solution was continuously aerated but not replaced during the treatment. Evaporated/transpired water was resupplied twice during the treatment. (mean±SD, n=3; values marked with different letters are significantly different at P<0.05)

The stomatal conductance was the same in H4 and H40 controls (Fig. 5). The SS treatments have a negative effect on the stomatal conductance showing a sharply decreasing trend with increasing doses.

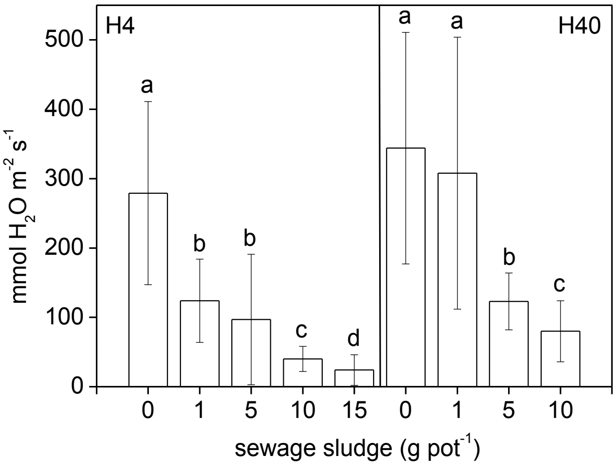

Figure 5. Stomatal conductance in the youngest fully developed leaves of Szarvasi-1 energy grass grown in H4 and H40 amended with 0, 1, 5, 10 and 15 g dried SS in 0.8 dm^3 pots. (mean±SD, n=3; values marked with different letters are significantly different at P<0.05)

The control plants grown in H4 and H40 had similar chlorophyll concentration in the youngest fully developed leaves, 1.79 and 1.83 mg Chl g^{-1} DW with a 2.9 and 3.0 Chl *a/b* ratio, respectively. There was no considerable effect of any SS treatments on Chl concentration and *a/b* ratio (2.9-3.1).

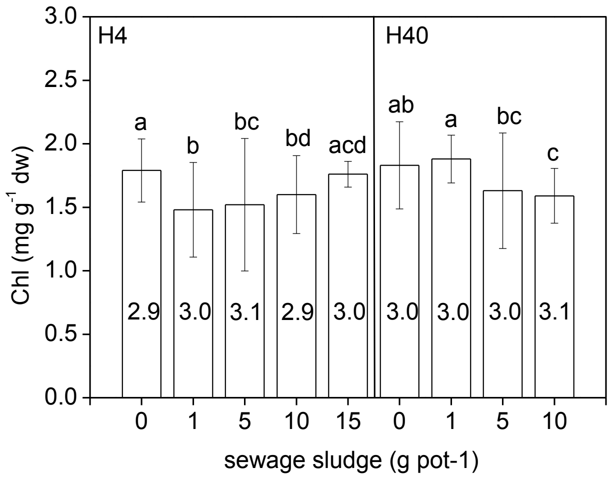

Figure 6. Chlorophyll concentration and Chl *a/b* ratio (indicated in each column) in the youngest fully developed leaves of Szarvasi-1 energy grass grown in H4 and H40 amended with 0, 1, 5, 10 and 15 g dried SS in 0.8 dm^3 pots. (dw=dry weight; mean±SD, n=3; values marked with different letters are significantly different at P<0.05)

Maximal quantum efficiency of PSII

The maximal quantum efficiency of PSII was not affected by the increasing SS treatment compared to the controls but 10 g SS in H40 significantly decreased it compared to the highest values in both H4 and H40 (Fig. 7).

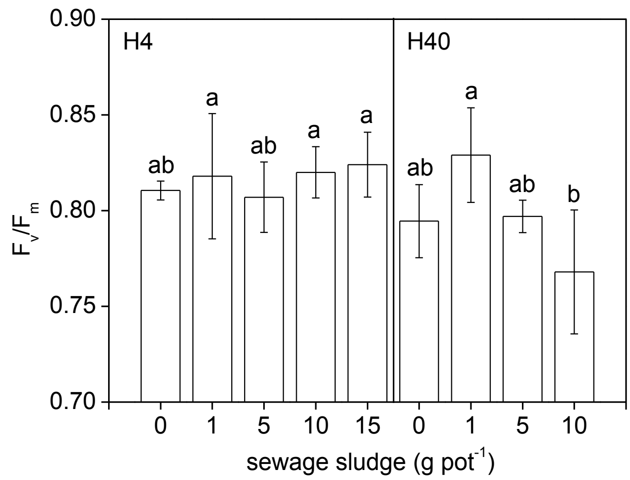

Figure 7. Maximal quantum efficiency of PS II in the youngest fully developed leaves of Szarvasi-1 energy grass grown in H4 and H40 amended with 0, 1, 5, 10 and 15 g dried SS in 0.8 dm^3 pots. (mean±SD, n=3; values marked with different letters are significantly different at P<0.05).

Metal uptake
Most of the elements had similar concentration in the shoots of control plants grown in H4 and H40 except for K and Zn which were higher in H4 (Table 2). Application of SS in the nutrient solution had a well-defined effect on the metal uptake of Szarvasi-1 energy grass. The majority of the elements shows a decreasing accumulation in the shoot as the SS doses increased in either the H4 or H40. These are the Ca, Mg, Mn, Zn, Cu, Cd. Some elements increased in the shoot: Na, Fe, Ni. Potassium decreased in the shoots of plants grown in H4 but increased in H40.

Discussion

SS added to nutrient solutions
The SS contains significant quantities of organic matter and inorganic elements that provides the possibility to use it as organic amendment to reverse the degradation of soil and to restore soil organic matter as well as supplying readily available nutrients to plants (Shaheen et al. 2014). For this reason, its application to agricultural soils is an economically attractive management strategy, but its use has been limited because SS often contains significant amounts of potentially toxic metals (Singh and Agrawal 2010). The total elemental composition of the dried SS applied in this study is typical of communal wastes (Fytili and Zabaniotou 2008, Singh and Agrawal 2008, Usman et al. 2012). It contains low concentration of heavy metals (Cu, Fe, Mn, Zn, Cr, Ni, Co, Pb) except for Cd which is a little higher as compared to typical metal content in SSs (Table 1)(Fytili and Zabaniotou, 2008). However, the organically bound metals in SS are less available than more mobile metal salts found in commercial fertilizers (Frost and Ketchum Jr. 2000). This was found when the dried SS was added to deionized water or nutrient solution. There was a moderate (1-5x) increase in the soluble concentration of most of the elements measured (Ca, K, Mg, S, P, B, Cu, Fe, Zn) and a great increase in that of Na. Moreover, the SS amended nutrient solution contained soluble Al, Cr, Li, Sr and Ti in low concentration. Mn concentration decreased compared to the unamended control solution while Cd, Co, Ni and Pb levels remained below detection limit although the dried SS contained them in a considerable concentration. These changes can be explained by the binding of metal cations to the organic matter in the studied SS (Amir et al. 2005). The initial pH of the unamended nutrient solutions

in this study was moving from the slightly acidic level towards neutral level by the addition of increasing amounts of SS. The increasingly alkaline pH may also contribute to the decreasing availability of some metal ions especially Fe (Marschner 1995).

Table 2. Metal concentrations in the shoots of Szarvasi-1 energy grass grown in H4 and H40 amended with 0, 1, 5, 10 and 15 g dried SS in 0.8 dm^3 pots. (mean±SD, n=3; values marked with different letters are significantly different at P<0.05)

SS (g pot^{-1})		0	1	5	10	15
K (µmol g-1 dw)	H4	1315±309 a	1154±86 b	949±79 c	857± 40 d	802±29 e
K (µmol g-1 dw)	H40	668± 22 a	659±88 a	844±83 a	828±132 a	
Ca (µmol g-1 dw)	H4	130± 22 a	105± 4 b	68±18 c	60± 1 d	54± 2 e
Ca (µmol g-1 dw)	H40	129± 8 a	118±21 b	51± 7 c	62± 20 c	
Mg (µmol g-1 dw)	H4	114± 8 a	126±12 a	87± 20 b	72± 4 c	60± 0 d
Mg (µmol g-1 dw)	H40	91± 28 a	100±21 a	77± 4 b	69± 16 c	
Na (µmol g-1 dw)	H4	13± 3 a	20± 4 b	27± 6 b	45± 8 c	49±1 c
Na (µmol g-1 dw)	H40	26± 19 a	39±22 a	30± 8 a	45± 13 a	
Fe (µmol g-1 dw)	H4	1.0±0.1 a	2.2±0.3 b	1.0±0.4 a	1.1±0.1 a	1.3±0.3 a
Fe (µmol g-1 dw)	H40	0.7±0.2 a	0.8±0.1 a	1.0±0.5 a	1.2±0.6 a	
Mn (µmol g-1 dw)	H4	2.3±1.0 a	2.2±0.2 a	1.3±0.4 bd	0.8±0.1 cd	0.8±0.1 c
Mn (µmol g-1 dw)	H40	1.8±0.2 a	1.7±0.2 a	1.3±0.1 ac	1.2±0.3 bc	
Zn (µmol g-1 dw)	H4	1.6±0.3 a	1.4±0.2 a	0.8±0.2 b	0.5±0.0 b	0.4±0.0 c
Zn (µmol g-1 dw)	H40	1.0±0.1 a	1.1±0.3 a	0.6±0.1 b	0.5±0.1 b	
Cu (µmol g-1 dw)	H4	0.14±0.02 a	0.17±0.02 a	0.07±0.01 b	0.06±0.01 b	0.05±0.01 b
Cu (µmol g-1 dw)	H40	0.11±0.02 a	0.12±0.02 a	0.06±0.01 b	0.06±0.01 b	
Cd (nmol g-1 dw)	H4	2.5±0.3 a	2.3±0.2 a	1.2±0.6 b	0.7±0.3 c	0.6±0.2 c
Cd (nmol g-1 dw)	H40	2.1±0.3 a	1.6±0.6 a	1.5±1.0 a	<DL	
Ni (nmol g-1 dw)	H4	4.1±2.6 a	6.5±1.8 a	8.7±5.4 a	11.0*	9.6±0.9 a
Ni (nmol g-1 dw)	H40	1.2±0.1 a	5.3±1.3 b	4.6±0.4 b	7.6±0.4 c	

DL, detection limit
*, only one datum available

Toxic effects of the SS treatments

The root and shoot growth was oppositely affected by the SS treatments in H4 (Figs. 1-3). While root biomass decreased, shoot biomass was largely unaffected but the shoot water content and shoot length decreased and root water content was not influenced. The toxic effects was exerted mostly by the two largest doses of the SS treatment while at the lowest dose root and shoot biomass and shoot length was even increased slightly. The majority of studies have reported positive effect of SS on the crop yield even in case of a high dose or a long term application (Singh and Agrawal, 2008). However, Qasim et al. (2001) found that at the highest amendment doses in field application the germination and yield had declined. The water content of the root tissues may have correlated with the availability of osmotically active solutes in the nutrient solution. Nevertheless, the parallel decrease in the root biomass and the shoot water content and length implicate that damages may have occurred in the roots that also inhibited the water uptake somewhat. Thus, the toxic effects influence the biomass production at the level of the physiology of roots. Similar changes were observed on the root and shoot biomass production of willow, where the nutrient supply was high enough to make willows decrease the root growth proportionally to the aboveground parts (Jerbi et al. 2015).

The pH of the nutrient solutions increased significantly during the 3 week growth period after amendment. Being a Pontic taxon that originates from carbonate-rich soils around the Black Sea region, Szarvasi-1 energy grass prefers alkaline pH (Csete et al. 2011) and this was shown in case of the control plants and the two lower treatment doses in which the pH reached 7.8. However, in the two higher treatment doses the pH further increased up to 8.5 which may reflect that the roots struggled with maintaining the required cytoplasmic homeostasis (Fig. 4).

Changes in the Chl concentration and Chl *a/b* ratio of the plants as well as the maximal quantum efficiency of PSII were not influenced by any doses of the SS treatments significantly (Figs. 6 and 7). This means that neither the composition nor the photochemical functioning of the photosynthetic apparatus suffered from any toxic or inhibitory effects. Nevertheless, the stomatal conductance (Fig. 5) decreased in parallel with the increasing SS concentration that could have inhibited the carbon assimilation and thus lead to a decreased biomass production. Since disturbances in the carbon assimilation do not affect the PSII quantum efficiency in general, this effect was not measured in the photochemical activity. Nevertheless no severe damages, such as increased ROS production occurred by the decreased stomatal conductance which could have led to the increase in the functioning of PSII reaction centres. The data are in agreement with the work of Mata-Gonzalez et al. (2002) who studied gas exchange and photosynthesis rate in desert grasses. In nodulated alfalfa plants subjected to cyclic drought, SS treatment also improved photosynthetic activity (Antolin et al. 2010).

Toxic elements accumulating in the shoot may be the reason for the toxic effects of high doses of SS. However, in our study, most of the toxic elements decreased with increasing SS doses such as Cd and Cu that have been proved to cause toxicity in Szarvasi-1 energy grass (Table 2) (Sipos et al. 2013). Cd is known to decrease the stomatal conductance by interacting with the Ca metabolism and thus disrupting the stomatal opening (Perfus-Barbeoch et al. 2002). Cd was also shown to alter the cell wall thickening, leading to stomatal dysfunctions (Vitória et al. 2003). The elements that were increasing with SS doses were Na, Fe and Ni. Even though plants have different sensitivity against high iron concentrations, iron toxicity may appear in plants at several orders of magnitude higher concentration than that measured in our plants (2 μmol g^{-1} dw). In rice plants iron toxicity manifested in the decrease of the grain yield was found over 200-400 mg Fe kg^{-1}, equal to around 3.5-7.0 mmol Fe kg^{-1} (Audebert and Sahravat, 2000). In sensitive species, sodium may cause decline in yield and disturbances in the water regime but also in a much higher concentration than measured here (50 μmol g^{-1} dw)(Munns 2002). Furthermore it was reported that Szarvasi-1 energy grass is well adapted to salinity (Csete et al. 2011). Ni may be another toxic metal once high concentrations reach the sensitive tissues. For Szarvasi-1 energy grass the shoot concentration resulting in decline in biomass and physiological parameters was 0.5 μmol g^{-1} dw (Sipos et al. 2013) whereas in this study its highest shoot concentration was 0.01 μmol g^{-1} dw.

As there was no clear symptom of metal toxicity even at the highest SS treatments such as decreased Chl concentration and photosynthetic activity we concluded that the main reason for decreases in the measured indices was i. the anchoring of essential metals in the organic components of the SS resulting in nutrient inbalance, ii. the cumulative and synergistic effect of non-essential elements, iii. the effect of toxic organic components that we did not measure.

Nutritive effects of the SS treatments

In H40 the root dry mass of control plants was almost double compared to that in H4 whereas the shoot dry mass was almost the same (Fig. 1). This may refer to the root growth stimulation by the nutrient deficiency (Hermans et al. 2006). Decreasing root biomass was observed with increasing rates of SS application until the control level of H40 was reached and simultaneously the shoot biomass was increasing showing the stimulating effect of increasing SS doses. Only the highest, 10 g per pot SS application resulted in a significant drop in root and shoot biomass indicating the toxicity of high-dose of the applied SS by metabolic disturbances in plants. Shoot length showed similar changes (Fig. 3). But the water content of the root and shoot showed a clear increasing tendency with increasing SS application rates (Fig. 2).

Parameters showing physiological changes also reflected stimulation or no effect up to 5 g SS application to H40 (Figs. 4-7). The highest applied dose, 10 g per pot resulted in extreme pH increase and decline in the PSII maximal quantum efficiency. Only stomatal conductance showed a decreasing tendency with increasing SS doses above 1 g per pot. This latter, however, corresponds to the increasing root and shoot water content. Similar effect was observed on *Larix decidua* seedlings, where SS application improved the net photosynthesis, elevated the chlorophyll content and enhanced the dry matter accumulation rate (Bourioug et al., 2015)

Based on the above observations the moderate doses of SS appear to have a good nutritive value. This is further confirmed by the shoot element concentrations. Toxic metals were detected in a lower concentration compared to the SS treatments in H4 while essential ones were at similar level in spite of the one tenth dilution of the original (H4) solution. Potassium and Fe showed increase as the SS dose increased that could highly contribute to the positive effect of the treatments.

Nutrient solution as a model system for testing SS

In conclusion, applying SS in nutrient solution has a combined effect: the concentration of plant available nutrients and other ions may increase with the SS element content but also decrease due to introduction of organic components with C.E.C. This may represent a quick model system for testing how the SS may alter the nutrient balance in the medium. In this study we have experienced the applied SS with low heavy metal content has a moderate toxic effect on Szarvasi-1 energy grass at the highest application rates. Stomatal conductance appeared to be the most sensitive parameter. While applying the SS in a solution with decreased nutrient content clearly showed the nutritive its value. Based on the present findings SS amended soil cultures will be further tested to improve degraded lands with SS amendments that would be utilized in renewable crop production for energy production. This application would avoid human and animal health problems and further reduce expenses emerging due to environmental issues.

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