AGROCHEMISTRY AND SOIL SCIENCE
67 (2018) 1, **91-103**
DOI: 10.1556/0088.2018.67.1.7

Long-term field fertilization experiment with energy willow (*Salix* sp.) – Elemental composition and chlorophyll fluorescence in the leaves

[1]László SIMON, [2]Marianna MAKÁDI, [1]György VINCZE, [1]Zsuzsanna URI, [1]Katalin IRINYINÉ OLÁH, [2]László ZSOMBIK, [1]Szabolcs VÍGH, [1]Béla SZABÓ

[1]Department of Agricultural Sciences and Environmental Management, Institute of Engineering and Agricultural Sciences, University of Nyíregyháza, Nyíregyháza, Hungary;
[2]Research Institute of Nyíregyháza, University of Debrecen Centre of Agricultural Sciences, Nyíregyháza, Hungary

Abstract

A small-plot long-term field fertilization experiment was set up in 2011 with willow (*Salix triandra* x *Salix viminalis* 'Inger') grown as an energy crop in Nyíregyháza, Hungary. The brown forest soil was treated three times (in June 2011, May 2013, May 2016) with municipal biocompost (MBC), municipal sewage sludge compost (MSSC) or willow ash (WA), and twice (June 2011, May 2013) with rhyolite tuff (RT). In late May – early June 2016 urea (U) and sulphuric urea (SU) fertilizers were also applied to the soil as top-dressing (TD). These fertilizers and amendments were also applied to the soil in 2016 in the combinations; MBC+SU, RT+SU, WA+SU and MSSC+WA. All the treatments were repeated four times. In July 2016 the highest nitrogen concentrations in willow leaves were measured in the U (3.47 m/m%) and SU (3.01 m/m%) treatments, and these values were significantly higher than the control (2.46 m/m%). An excess of nitrogen considerably reduced the Zn uptake of the leaves, with values of 39.5 $\mu g\ g^{-1}$ in the U treatment, 53.4 $\mu g\ g^{-1}$ in the SU treatment, and 63.5 $\mu g\ g^{-1}$ in the control. All other amendments or TDs, except for WA, enhanced the specific potassium concentrations in willow leaves compared to the control. No significant quantities of toxic elements (As, Ba, Cd, Pb) were transported from soil amendments or TDs to the willow leaves. In July 2016 the most intensive leaf chlorophyll fluorescence was observed in the MSSC and MSSC+WA treatments.

Keywords: energy willow (*Salix* sp.), fertilization, long-term field experiment, leaf elemental composition, chlorophyll fluorescence

---

Postal address: LÁSZLÓ SIMON, University of Nyíregyháza, Institute of Engineering and Agricultural Sciences, Department of Agricultural Sciences and Environmental Management, H-4400 Nyíregyháza Sóstói út 31/b., Hungary.
E-mail: simon.laszlo@nye.hu

Introduction

The depletion of easily exploitable stocks of fossil fuels, the steadily increasing emission of carbon dioxide into the atmosphere from the burning of fossil fuels, and the danger of global warming have focused attention on energy production from biomass. The burning of biomass from 'energy' crops could significantly mitigate the anthropogenic emission of carbon dioxide and could partially replace fossil fuels. It was estimated that 1 hectare of energy crop biomass could eliminate approximately 5 tons of fossil-carbon emission (BLASKÓ, 2008; GRAHAM et al., 1992). Herbaceous (e.g. *Agropyron, Arundo, Sida*) or woody (e.g. *Populus, Robinia, Salix* etc.) plant species that are primarily cultivated in plantations for biomass production and energetical utilisation are considered to be energy crops (BLASKÓ, 2008).

Among the energy crops cultivated for their high aboveground biomass, rapidly growing willow (*Salix*) species are very promising in Hungary. Their harvestable fresh shoot biomass may be as much as 20 or 25 tons per hectare (or 10-12 tons of dry matter) annually. Willow sprouts well; its 2-4 m long shoots can be harvested every 1, 2 or 3 years. Short rotation coppice (SRC) energy plantations can be cultivated for 15-20 years in the same field, but balanced, regular nutrient supplies are required in the soil to achieve good aboveground biomass yields (GYURICZA et al., 2008; GYURICZA, 2011; SIMON, 2011; SMART & CAMERON, 2012).

The biomass yield of energy crops can be improved by the application of various inorganic or organic fertilizers and additives to the soil, including biowastes (e.g. sewage sludge) and wood ash, and also by municipal wastewater irrigation (DIMITRIOU et al., 2006; GYURICZA, 2011; PARK et al., 2005; SMART & CAMERON, 2012; SIMON et al., 2012ab, 2013a, 2016). Enhanced growth is usually observed in response to fertilization, when *Salix* sp. are cultivated in SRC plantations, but in some cases the effect is less pronounced. This variation could be due to the nutrient status of the soil, while the type and availability of the nutrients in the fertilizer may also be important. The nitrogen status of the soil is thought to be crucial (SEVEL et al., 2014a,b).

However, the application of various amendments (e.g. municipal sewage sludge or wood ash) to the soil may enhance not only the uptake of beneficial elements (e.g. nitrogen or potassium), but also the accumulation of toxic elements in willow organs (DIMITRIOU et al., 2006; GYURICZA et al., 2008; PARK et al., 2005). This will then be reflected in the toxic metal concentration of the harvested shoots and that of the ash after biomass burning. The Cd or Zn uptake rates of *Salix* sp. are high compared to other plant species and to other trace elements (BERNDES et al., 2004; UTMAZIAN et al., 2007, DICKINSON & PULFORD, 2005; VYSLOUŽILOVÁ et al., 2003). Therefore, from the phytoremediation point of view, SRC willow could be useful for the removal of metal contaminants from the soil (PULFORD & DICKINSON, 2006).

Since most of the SRC plantations in Hungary were established during the last fifteen years, insufficient information is available as yet on how mineral fertilizers, biowastes and inorganic soil amendments affect the mineral nutrition and growth rate of bioenergy crops. The aim was to compare the transport (uptake or accumulation) rates of various mineral nutrients and potentially toxic elements from soil fertilizers and amendments to the leaves of willow. Chlorophyll fluorescence yields in willow leaves were also studied to compare the ecophysiological responses of willow to various fertilizers and amendments.

The assumption was that the mineral nutrients in fertilizers and amendments would have different effects on the uptake or accumulation rates of macro, micro or toxic elements in the leaves, leading to a stimulating or inhibitory effect on chlorophyll fluorescence yields.

Materials and Methods

A small-plot long-term field experiment with energy willow (*Salix triandra* x *Salix viminalis* cv. Inger) was set up in April 2011 in the experimental field of the Research Institute of Nyíregyháza, Centre of Agricultural Sciences, University of Debrecen. In each 27 m^2 plot 40 willow bushes are grown with 0.75 m row spacing and 0.6 m between plants. In every small plot plants are grown in two twin rows with 1.5 m spacing. The 0-25 cm layer of the uncontaminated brown forest soil had the following characteristics: loamy sand texture; pH_{H2O} 8.10, pH_{KCl} 7.52, $CaCO_3$ 4.80 m/m%, total salt content <0.02 m/m%, humus 1.51%, CEC 10.4 cmolc/kg; P–713, K–5653, Ca–21773, Mg–5471, Cu–12.7, Mn–653, Zn–44.3, As–38.3, Cd–0.11, Pb–13.6 mg kg^{-1} in HNO_3–H_2O_2 extract. The plant-available nutrient concentrations (mg kg^{-1}) of the soil were the following: NH_4-N: 5.68, NO_3-N: 6.37, AL-P_2O_5: 302, AL-K_2O: 251, KCl-Mg: 425, KCl-SO_4-S: 5.87, EDTA-Cu: 2.89, EDTA-Fe: 25.3, EDTA-Mn: 60.4, EDTA-Zn: 2.94, AL-Na: 44.3 (SIMON et al., 2012b).

The pH and lime content of the soil is unusually high, since its surface was covered during the 1930s with drainage canal dredging sludge.

The experiment was set up in 2011 on a total area of 3800 m^2 in a random block design on 40 small plots with 10 treatments in 4 replications. During April and May 2016 the soil treatments were the following:

1. *Control* (no fertilization since 2011).
2. *Urea* (U) – 100 kg ha^{-1} dry weight with 46% nitrogen, applied as top-dressing[1].
3. *Sulphuric urea* (SU) – 100 kg ha^{-1} dry weight with 46% nitrogen applied as top-dressing[2].

---

[1] During spring (May or June) of 2011, 2012, 2013, 2014 and 2015 these plots received 100 kg ha^{-1} ammonium nitrate (34% N) as top-dressing.

[2] These plots were formerly treated (during May or June of 2011, 2012, 2013) with 100 kg ha^{-1} ammonium nitrate (34% N), then in May 2014 and early June 2015 with 100 kg ha^{-1} of urea (46% N) as top-dressing.

4. *Municipal biocompost* (MBC) – 20 t ha^{-1} wet weight with 75-76% dry matter (also previously applied in June 2011 and May 2013).

5. *Municipal sewage sludge compost* (MSSC) – 15 t ha^{-1} wet weight with 48-56% dry matter (producer Nyírségvíz Ltd., Nyíregyháza, Hungary; also previously applied in June 2011 and May 2013).

6. *Rhyolite tuff* (RT) – 30 t ha^{-1} wet weight with 18% moisture content (producer Colas-Északkő Bányászati Ltd., Tarcal, Hungary; applied in June 2011 and May 2013, but not in 2016).

7. *Willow ash* (WA) – 300 kg ha^{-1} dry weight with 1% moisture content (produced at the University of Nyíregyháza by burning willow shoots without leaves; also applied in June 2011 and May 2013 at a rate of 600 kg ha^{-1}).

8. *MBC + SU* top-dressing
9. *MSSC + SU* top-dressing
10. *WA + SU* top-dressing
11. *MSSC + WA*.

The above fertilizers and additives were immediately rotated into the upper 0-25 cm layer of the soil. The basic physical and chemical characteristics and plant nutrient content of municipal biocompost, municipal sewage sludge compost, rhyolite tuff and willow ash were the following:

– *Municipal biocompost:* pH_{H2O} 7.72, pH_{KCl} 7.23, $CaCO_3$ 3.56 m/m%, total salt content 0.83 m/m%, total C 6.93 m/m %, total N 0.83 m/m %, NH_4-N 18.4 mg kg^{-1}, NO_3-N 115 mg kg^{-1}; P–2259, K–6721, Ca–22551, Mg–3199, Fe–10958, Cu–44.7, Mn–317, Zn–148, As–6.26, Cd–0.40, Pb–23.5 mg kg^{-1} in HNO_3–H_2O_2 extract; P_2O_5–2812, K_2O–5109, Ca–16967, Mg–1311, Fe–1579, Cu–19.4, Mn–205, Zn–27.4, As–2.24, Cd–0.25, Pb–15.9 mg kg^{-1} in Lakanen-Erviö extract.

– *Municipal sewage sludge compost:* pH_{H2O} 5.93, pH_{KCl} 5.91, $CaCO_3$ 0 m/m%, total salt content 3.34 m/m%, total C 10.4 m/m %, total N 1.84 m/m %, NH_4-N 169 mg kg^{-1}, NO_3-N 42.3 mg kg^{-1}; P–18876, K–3424, Ca–39294, Mg–4479, Fe–17149, Cu–226; Mn–390, Zn–822, As–20.7, Cd–1.38, Pb–49.4 mg kg^{-1} in HNO_3–H_2O_2 extract; P_2O_5–12269, K_2O–1653, Ca–21761, Mg–1839, Fe–5038, Cu–94, Mn–229, Zn–577, As–6.72, Cd–0.76, Pb–21.1 mg kg^{-1} in Lakanen-Erviö extract.

– *Rhyolite tuff:* pH_{H2O} 7.44, pH_{KCl} 6.40, $CaCO_3$ 0.08 m/m%, total salt content <0.01 m/m%, humus 0%, NH_4-N 9.36 mg kg^{-1}, NO_3-N 7.90 mg kg^{-1}; P–28.4, K–1871, Ca–1140, Mg–455, Fe–5738, Cu–2.34, Mn–43.1, Zn–51.1, As–1.22, Cd–0.074; Pb–23.5 mg kg^{-1} in HNO_3–H_2O_2 extract; P_2O_5–4.29, K_2O–133, Ca–974, Mg–133, Fe–8.05, Cu–1.22, Mn–4.94, Zn–0.90, As–not detected, Cd–0.019, Pb–0.375 mg kg^{-1} in Lakanen-Erviö extract.

– *Willow ash:* pH_{H2O} 10.9, pH_{KCl} 10.7, total salt content 1.17 m/m%, NH_4-N 0 mg kg^{-1}, NO_3-N 0 mg kg^{-1}; P–6472, K–16508, Ca–43074, Mg–7991, Fe–17045, Cu–546, Mn–624, Zn–279, As–35.4, Ba–129, Cd–0.36, Pb–9.42 mg kg^{-1} in HNO_3–H_2O_2 extract; P_2O_5–5218, K_2O–10955, Ca–35432, Mg–3874, Fe–422, Cu–11.8, Mn–328, Zn–164, As–9.92, Ba–not detected, Cd–0.134, Pb–2.49 mg kg^{-1} in Lakanen-Erviö extract.

All the above data were the means of 3 measurements from combined samples, prepared from 30 subsamples for all the substances investigated. The methods were described in SIMON et al. (2013a,b; 2015).

Salix leaves were sampled 9-11 weeks after the application of the soil amendments or the urea or sulphuric urea top-dressing on July 6, 2016. Fully developed leaves were collected from the 30-60 cm uppermost section of the shoots from 20 plants per plot in 4 replicates. The macro- and microelement concentrations were determined in mixed, washed, dried and ground plant samples using the ICP-OES technique after HNO_3–H_2O_2 digestion. The nitrogen concentration of the samples was determined with the Kjeldahl method. All chemical analyses were done in 4 replicates (SIMON et al., 2012b, 2013a, 2015).

Leaf fluorescence measurements were conducted by July 22, 2016 with an OS5p chlorophyll fluorescence meter (Opti-Sciences, Inc., USA), which measures the quantum yield after the emission of excitation light on the leaf surface. The measurements were done on fully developed leaves from the 30-60 cm uppermost section of the shoots. Two bushes were chosen from the middle of every second plot, and measurements were made on five shoots per bush, giving 10 replications per plot. The leaf fluorescence measurements were made in two independent replications for each treatment.

The data were analysed with SPSS 18.0 software using analysis of variance (ANOVA) followed by treatment comparison using Tukey's b-test or Tukey's HSD-test.

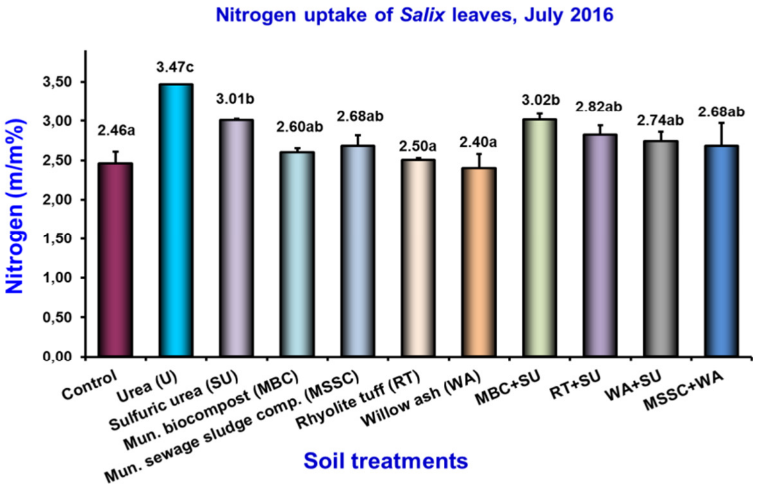

Figure 1

Nitrogen uptake of *Salix* leaves 9-11 weeks after the application of soil amendments or urea or sulphuric urea top-dressing (July 6, 2016). Data are the means of 4 replications. ANOVA, Tukey's b-test: means within columns followed by the same letter are not significant at $P<0.05$.

Results and Discussions

The influence of the soil amendments and of urea and sulphuric urea fertilizers as top-dressing on the nitrogen concentration of the willow leaves is shown in *Figure 1*.

The application of a moderate dose of urea in May significantly enhanced the nitrogen uptake of willow leaves in July, compared to all the other treatments. The application of sulphuric urea or MBC+SU also had a significant enhancing effect on nitrogen uptake compared to the control, rhyolite tuff and willow ash treatments. It is well documented that willow plants respond well to nitrogen fertilization, and that the enhanced uptake of nitrogen by the leaves or shoots increases the harvestable shoot yield (Weih & Rönnberg-Wästljung, 2007; Simon et al., 2016; Merilo et al., 2006).

Since the RT and WA soil amendments contained no nitrogen, this explains why the lowest nitrogen concentrations were recorded in these treatments and in the control.

Table 1

Concentration of macroelements in willow leaves 9-11 weeks after the application of soil amendments or top-dressing (July 6, 2016)

Treatments	P	K	Ca	Mg	S
	mg g^{-1} dry matter				
Control	3.557^{a}	11.874^{ab}	10.132^{a}	5.352^{a}	3.489^{a}
Urea (U)	3.007^{a}	16.136^{c}	12.651^{a}	5.252^{a}	4.464^{ab}
Sulphuric urea (SU)	3.073^{a}	14.593^{bc}	11.762^{a}	5.280^{a}	4.563^{ab}
Municipal biocompost (MBC)	3.077^{a}	13.926^{abc}	10.734^{a}	4.964^{a}	4.035^{ab}
Municipal sewage sludge compost (MSSC)	3.467^{a}	13.064^{abc}	11.568^{a}	4.892^{a}	4.942^{b}
Rhyolite tuff (RT)	3.275^{a}	13.048^{abc}	10.786^{a}	4.854^{a}	3.702^{ab}
Willow ash (WA)	3.240^{a}	11.113^{a}	9.814^{a}	5.315^{a}	3.787^{ab}
MBC+SU	3.286^{a}	16.290^{c}	10.896^{a}	5.266^{a}	4.758^{ab}
RT+SU	3.041^{a}	14.504^{bc}	11.585^{a}	4.817^{a}	4.325^{ab}
WA+SU	2.945^{a}	13.794^{abc}	10.944^{a}	4.985^{a}	4.048^{ab}
MSSC+WA	3.618^{a}	14.881^{bc}	10.489^{a}	5.198^{a}	4.735^{ab}

Data are the means of 3 replications. ANOVA, Tukey's b-test: means within columns followed by the same letter are not significant at $P<0.05$.

Table 1 shows the concentration of *macroelements* in willow leaves 9-11 weeks after the application of soil amendments or top-dressing. The phosphorus concentration was not significantly influenced; the highest values were measured in the MSSC+WA treatment and the control and the lowest values in the WA+SU and U treatments. The highest leaf concentrations of potassium were recorded after urea or MBC+SU treatment and the lowest in the WA treatment. Although there were differences in the calcium and magnesium concentrations of the leaves, these were not significant. The highest sulphur concentration was observed in the MSSC treatment, which was significantly higher than in the control. The results confirm earlier findings that the application of various fertilizers and amendments may influence the uptake of macroelements in the shoots of willow (DIMITRIOU et al., 2006; PARK et al., 2005; SIMON et al., 2016).

Table 2 demonstrates the concentration of *essential microelements* in the willow leaves. The highest iron concentration was detected in the urea treatment and the lowest in the MSSC treatments, but the differences were not significant for either Fe, Cu or Mn. However, the additives and fertilizers resulted in significantly different zinc contents in the leaves, with the lowest concentration in the U treatment and the highest in the MSSC treatment. Among the soil amendments (MBC, MSSC, RT and WA), MSSC had the highest Zn concentration (475 mg kg^{-1}) (SIMON et al., 2012a, 2015), which explains the high rate of Zn transport to willow leaves.

Table 2

Concentration of essential microelements in the leaves of willow 9-11 weeks after the application of soil amendments and top-dressing (July 6, 2016)

Treatments	Fe	Cu	Mn	Zn
	µg g^{-1} dry matter			
Control	80.5^{a}	11.2^{a}	45.0^{a}	63.5^{abc}
Urea (U)	102.6^{a}	6.9^{a}	65.4^{a}	39.5^{a}
Sulphuric urea (SU)	87.4^{a}	8.9^{a}	69.8^{a}	53.4^{abc}
Municipal biocompost (MBC)	69.9^{a}	7.5^{a}	62.3^{a}	52.3^{abc}
Municipal sewage sludge compost (MSSC)	70.0^{a}	10.9^{a}	58.8^{a}	74.3^{c}
Rhyolite tuff (RT)	81.1^{a}	8.6^{a}	62.0^{a}	68.5^{bc}
Willow ash (WA)	78.2^{a}	8.7^{a}	70.2^{a}	57.1^{abc}
MBC+SU	92.5^{a}	9.9^{a}	50.0^{a}	63.3^{abc}
RT+SU	85.7^{a}	9.2^{a}	58.2^{a}	60.5^{abc}
WA+SU	84.1^{a}	7.6^{a}	61.3^{a}	47.5^{ab}
MSSC+WA	73.4^{a}	8.1^{a}	47.6^{a}	66.4^{bc}

Data are the means of 3 replications. ANOVA, Tukey's b-test: means within columns followed by the same letter are not significant at $P<0.05$.

Table 3 shows the concentration of selected *toxic elements* in willow leaves. The arsenic and lead concentrations were below the detection limit. The lowest barium concentration was detected in the control and the highest in the SU treatment. It is well-documented that willow species accumulate a higher concentration of cadmium in their shoots than other tree species, so *Salix* sp. are suitable for the phytoextraction of Cd-contaminated soils (BERNDES et al., 2004; DICKINSON & PULFORD, 2005; PULFORD & DICKINSON, 2006). In the present experiment the Cd concentrations ranged from 0.419–0.821 µg g^{-1} dry matter in the leaves, and these moderately low concentrations were not significantly influenced by the soil treatments. The highest Cd concentration (0.821 µg g^{-1} dry matter) was measured in the control while all the soil treatments resulted in lower rates of Cd accumulation. Similar Cd concentrations were observed both in September 2011 (SIMON et al., 2012b; 2013a), during the first leaf analysis after the first application of soil additives and fertilizers, and in July 2013 (SIMON et al., 2016), in the second leaf analysis, conducted after the second application of soil additives and fertilizers.

Table 3

Concentration of selected toxic elements in willow leaves 9-11 weeks after the application of soil amendments and top-dressing (July 6, 2016)

Treatments	As	Ba	Cd	Pb
	µg g^{-1} dry matter			
Control	b.d.l.	3.44^a	0.821^a	b.d.l.
Urea (U)	b.d.l.	4.78^a	0.419^a	b.d.l.
Sulphuric urea (SU)	b.d.l.	10.0^a	0.498^a	b.d.l.
Municipal biocompost (MBC)	b.d.l.	4.06^a	0.496^a	b.d.l.
Municipal sewage sludge compost (MSSC)	b.d.l.	4.23^a	0.620^a	b.d.l.
Rhyolite tuff (RT)	b.d.l.	3.72^a	0.758^a	b.d.l.
Willow ash (WA)	b.d.l.	3.80^a	0.621^a	b.d.l.
MBC+SU	b.d.l.	4.94^a	0.649^a	b.d.l.
RT+SU	b.d.l.	4.11^a	0.616^a	b.d.l.
WA+SU	b.d.l.	4.31^a	0.473^a	b.d.l.
MSSC+WA	b.d.l.	6.70^a	0.713^a	b.d.l.

Data are the means of 3 replications. ANOVA, Tukey's b-test: means within columns followed by the same letter are not significant at $P<0.05$. b.d.l.=below the detection limit: As-0.43 µg g^{-1}, Pb-0.30 µg g^{-1}.

Chlorophyll fluorescence is the light re-emitted by chlorophyll molecules when returning from the excited to the non-excited state. Chlorophyll fluorescence is used as an indicator of photosynthetic energy conversion in algae, bacteria and higher plants. Excited chlorophyll dissipates the absorbed light energy by driving photosynthesis, as heat in non-photochemical quenching, or by emission as fluorescence radiation. The chlorophyll fluorescence emitted from plant leaves gives an insight into the health of the photosynthetic systems within the leaf. Chlorophyll fluorimeters are designed to measure the fluorescence of photosystem II (PS-II). This fluorescence varies with plant stress, so it can be used to measure the level of stress intensity. In plants, the photosynthetic apparatus is very sensitive to the toxicity of various metals, which affects the assimilation process both directly, by inhibiting enzyme activity in the Calvin cycle, and indirectly, by inducing CO_2 deficiency at carboxylation sites due to stomatal closure (SHAH et al., 2010; BERNARDINI et al., 2016; ŻUREK et al., 2014; WIKIPEDIA CONTRIBUTORS, 2018a, 2018b).

Chlorophyll fluorescence was measured in the leaves of willow 11-13 weeks after the soil treatments. *Figure 2* shows the PS-II fluorescence yields (Y) in the leaves of *Salix* plants.

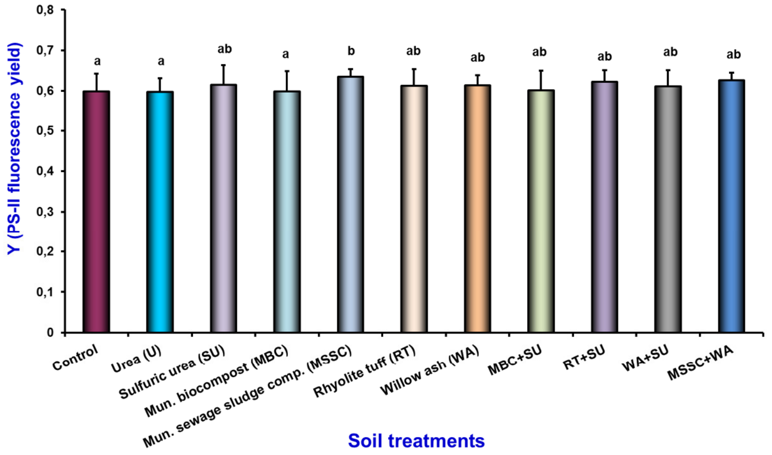

Figure 2

Effect of soil amendments and top-dressing on the leaf chlorophyll fluorescence yields of *Salix* (July 22, 2016). Data are the means of 2 independent and 10 internal replications per treatment. n=20. ANOVA, Tukey's HSD-test: means within columns followed by the same letter are not significant at $P<0.05$.

Comparing all the soil treatments, only MSSC application enhanced significantly the value of Y compared to the control, urea and MBC treatments. The fact that the highest rate of chlorophyll fluorescence was observed after MSSC treatment may be related to the good macro- and micronutrient supplies to the leaves (*Figure 1*, *Tables 1-2*), and suggests that the highest rate of photosynthesis and biomass shoots will be obtained with MSSC (WEIH & RÖNNBERG-WÄSTLJUNG, 2007; MERILO et al., 2006). This was confirmed in 2013 and 2016, when shoots (without leaves) were harvested for the first and second time, and previous treatment with MSSC was found to significantly enhance the harvested shoot weight compared with the control (SIMON et al., 2017).

It is advantageous that none of the soil treatments depressed chlorophyll fluorescence, compared to the control. It was reported by ŻUREK et al. (2014) that an excess of metals in leaves influences the level of chlorophyll fluorescence, but although the highest Zn concentrations were measured in the MSSC treatment (*Table 2*), the value of Y was not diminished. BERNARDINI et al. (2016) studied the ecophysiological responses to Zn stress in various *Salix* clones and found that the addition of low Zn concentrations (300 mg kg^{-1}) to the soil, below the phytotoxic limit, did not alter the gas exchange or photochemical activity of willow leaves. This was confirmed in the present work.

Conclusions

In summary, it can be concluded that all the additives and mineral fertilizers applied to the soil had a smaller or greater effect on the mineral nutrition of energy willow. The highest nitrogen concentration in the leaves was measured after soil application of urea or sulphuric urea as top-dressing, or after the combined soil application of municipal biocompost and sulphuric urea (MBC+SU). Most of the soil treatments had a significant effect on the potassium and sulphur uptake of willow leaves. The application of urea or MBC+SU resulted in the highest potassium concentrations in the leaves, while the lowest potassium concentration was found in the WA treatment. The highest sulphur concentration was observed in the MSSC treatment, which was significantly higher than in the control. All the treatments except MSSC reduced the copper and zinc concentrations in willow leaves, with the lowest amounts in the urea treatment. Since almost all the former treatments resulted in higher harvestable wood biomass than the untreated control (SIMON et al., 2017), this phenomenon could be attributed to the ‘dilution effect’, and to the well-known nitrogen–copper and nitrogen–zinc uptake antagonism (KABATA-PENDIAS & PENDIAS, 2001) in plant organs. There is no direct danger of toxic element accumulation (As, Ba, Cd, or Pb) in willow leaves from the soil additives or fertilizers tested. All the treatments reduced the Cd concentration in the leaves compared to the control; this could again be explained by the ‘dilution effect’ of the higher leaf biomass or by cadmium–zinc uptake antagonism (KABATA-PENDIAS & PENDIAS, 2001).

Among the soil treatments, only MSSC enhanced significantly the chlorophyll fluorescence in willow leaves, compared to the control, urea or MBC treatments. The high rate of chlorophyll fluorescence after MSSC treatment may be related to the good macro- and micronutrient supplies to the leaves, and suggests that this treatment will result in the highest rate of photosynthesis and the highest biomass accumulation in the shoots.

Acknowledgements

This research was supported by Nitrogénművek Vegyipari Co. (Pétfürdő, Hungary), who provided the urea and sulphuric urea, and by Colas-Északkő Ltd. (Tarcal, Hungary), who provided the rhyolite tuff. The licence holder of the studied willow species is Lantmännen Agroenergi AB, Sweden (Hungarian distributor Holland-Alma Ltd., Piricse).

References

BERNARDINI, A., SALVATORI, E., DI RE, S., FUSARO, L., NERVO, G., MANES, F., 2016. Natural and commercial *Salix* clones differ in their ecophysiological response to Zn stress. Photosynthetica. **54**. (1) 56–64.

BERNDES, G., FREDRIKSON, F., & BÖRJESSON, P., 2004. Cadmium accumulation and *Salix*-based phytoextraction on arable land in Sweden. Agriculture, Ecosystems & Environment. **103**. (1) 207–223.

BLASKÓ, L., 2008. Cultivation of energy plants, site suitability, availability (in Hungarian). In: Renewable Agriculture. (ed.: CHLEPKÓ, T.). 167–207. Magyar Katolikus Rádió. Budapest.

DICKINSON, N.M. & PULFORD, I.D., 2005. Cadmium phytoextraction using short-rotation coppice *Salix*: the evidence trail. Environ. Int. **31**. (4) 609– 613.

DIMITRIOU, I., ERIKSSON, J., ADLER, A., ARONSSON, P., & VERWIJST, T., 2006. Fate of heavy metals after application of sewage sludge and wood-ash mixtures to short-rotation willow coppice. Environ. Pollut. **142**. (1) 160–169.

GRAHAM, R. L., WRIGHT, L. L., & TURHOLLOW, A. F.., 1992. The potential for short-rotation woody crops to reduce U.S. CO_2 emissions. Clim. Change. **22**. (3) 223–238.

GYURICZA, C., NAGY, L., UJJ, A., MIKO, P., & ALEXA, L., 2008. The impact of composts on the heavy metal content of the soil and plants in energy willow plantations (*Salix* sp.). Cereal Res. Commun. **36**. (Supplement 5) 279–282.

GYURICZA, C. 2011. Cultivation of woody energy crops (5.). Plant nutrition in energy plantations (in Hungarian). Agrofórum. **March 2011**. 92–96.

KABATA-PENDIAS, A. & PENDIAS, H., 2001. Trace Elements in Soils and Plants (3rd edition). CRC Press LLC. Boca Raton, London, New York, Washington D.C.

MERILO, E., HEINSOO, K., KULL, O., SÖDERBERGH, I., LUNDMARK, T., & KOPPEL, A., 2006. Leaf photosynthetic properties in a willow (*Salix viminalis* and *Salix dasyclados*) plantation in response to fertilization. Eur. J. Forest Res. **125**. (2) 93–100.

PARK, B. B., YANAI, R. D., SAHM, J. M., LEE, D. K., & ABRAHAMSON, L. P., 2005. Wood ash effects on plant and soil in a willow bioenergy plantation. Biomass Bioenergy. **28**. (4) 355–365.

PULFORD, I.D. & DICKINSON, N.M., 2006. Phytoremediation technologies using trees. In: Trace Elements in the Environment. Biogeochemistry, Biotechnology, and Bioremediation. (eds.: PRASAD, M.N.V., SAJWAN, K.S., NAIDU, R.). 383–403. CRC Press. Taylor and Francis Group. Boca Raton. Florida.

SEVEL, L., NORD-LARSEN, T., INGERSLEV, M., JØRGENSEN, U., & RAULUND-RASMUSSEN, K., 2014a. Fertilization of SRC willow, I: Biomass production response. Bioenerg. Res. **7**. 319–328.

SEVEL, L., INGERSLEV, M., NORD-LARSEN, T., JØRGENSEN, U., HOLM, P. E., SCHELDE, K., & RAULUND-RASMUSSEN, K., 2014b. Fertilization of SRC willow, II: Leaching and element balances. Bioenerg. Res. **7**. 338–352.

SHAH, F. U. R., AHMAD, N., MASOOD, K. R., & PERALTA-VIDEA, J. R., 2010. Heavy metal toxicity in plants. In: Plant Adaptation and Phytoremediation. (eds.: ASHRAF, M., AHMAD, M. S. A., & OZTURK, M.) 71–97, Springer Science+Business Media B.V.

SIMON, L. (ed.). 2011. Biomass utilisation for energy production in Észak-Alföld Region (Hungary) (in Hungarian and English). Bessenyei Book Publisher. Nyíregyháza (Hungary). 1–114.

SIMON, L., VINCZE, G., VARGA, C., SZABÓ, B., & KONCZ, J., 2012a. Passive phytoextraction of toxic elements from sewage sludge compost by *Salix viminalis* energy plants. Acta Phytopath. Entomol. Hung. **47.** (2) 285–291.

SIMON L. SZABÓ B., VINCZE G., VARGA C., SZABÓ M., KONCZ J., 2012b. Effect of ammonium nitrate fertilizer and soil additives on the mineral nutrition of energy willow (*Salix viminalis* L.) energy plants (in Hungarian). In: 1st Soil Science, Water Management and Crop Science Day. 'Soil-Water-Plant Relationship in Plant Production Space'. Debrecen, Hungary. November 23, 2012. (ed.: LEHOCZKY É.). 127–130. Szent István University Publisher. Gödöllő.

SIMON, L., SZABÓ, B., SZABÓ, M., VINCZE, GY., VARGA, C., URI, Z., & KONCZ, J., 2013a. Effect of various soil amendments on the mineral nutrition of *Salix viminalis* and *Arundo donax* energy crops. Eur. Chem. Bull. **2.** (1) 18–21.

SIMON, L. MAKÁDI, M., VINCZE, G., SZABÓ, B., SZABÓ, M., ARANYOS, T., 2013b. Impact of ammonium nitrate and rhyolite tuff soil application on the photosynthesis and growth of energy willow. In: International Multidisciplinary Conference. 10th edition. May 22-24, 2013. Baia Mare, Romania – Nyíregyháza, Hungary. (eds.: UNGUREANU, N., COTETIU, R., SIKOLYA, L., PÁY, G.). Scientific Bulletin, Serie C, Fascicle: Mechanics, Tribology, Machine Manufacturing Technology. 143-146. Bessenyei Book Publisher. Nyíregyháza (Hungary).

SIMON L. SZABÓ M., VINCZE G., URI Z., IRINYINÉ OLÁH K., MAKÁDI M., VÍGH S., 2015. Examination of nutritional supply of energy and arable crops, with particular reference to the combined effect of nitrogen fertilizers, biowastes and soil additives (in Hungarian). Research Final Report prepared for Nitrogénművek Vegyipari Co. (Pétfürdő, Hungary) on behalf of Nyír-Inno-Spin Ltd. (Nyíregyháza, Hungary). College of Nyíregyháza. 1–123. (manuscript).

SIMON L. VINCZE G., URI Z., IRINYINÉ OLÁH K., VÍGH S., MAKÁDI M., ARANYOS T., ZSOMBIK L., 2016. Long-term open-field fertilization experiment with energy willow (*Salix* sp.) – experiences of the first 5 years (in Hungarian). Növényterm. **65**. (2) 59–76.

SIMON L., URI Z., VINCZE G., IRINYINÉ OLÁH K., VÍGH S., 2017. Impact of artificial fertilizers, biowastes and mineral fertilizers on yield parameters of energy willow (*Salix* sp.) (in Hungarian). In: Indigenous and Landscape Varieties – Ecological Products – Healthy Nutrition – Rural Development Conference. University of Nyíregyháza, Nyíregyháza October 5-7. 2016. (ed. C. TÓTH). Proceedings of the Conference. Publisher: Technical and Agricultural Institute of the University of Nyíregyháza, Nyíregyháza, pp. 63-73. (ISBN 978-615-5545-69-6).

SMART, B.L. & CAMERON, K.D., 2012. Shrub willow. In: Handbook of Bioenergy Crop Plants. (eds.: KOLE, C., JOSHI, C. P. & SHONNARD, D.R.) 687–708. CRC Press. Boca Raton, London, New York.

UTMAZIAN, M. N. D. S., WIESHAMMER, G., VEGA, R., & WENZEL, W. W., 2007. Hydroponic screening for metal resistance and accumulation of cadmium and zinc in twenty clones of willows and poplars. Environ. Pollut. **148**. (1) 155–165.

VYSLOUŽILOVÁ, M., TLUSTOŠ, P. & SZÁKOVÁ, J., 2003. Cadmium and zinc phytoextraction of seven clones of *Salix* spp. planted on heavy metal contaminated soils. Plant Soil Environ. **49**. (12) 542–547.

WEIH, M. & RÖNNBERG-WÄSTLJUNG, A-N., 2007. Shoot biomass growth is related to the vertical leaf nitrogen gradient in *Salix* canopies. Tree Physiol. **27**. (11) 1551–1559.

WIKIPEDIA CONTRIBUTORS, 2018a. Chlorophyll fluorescence. In Wikipedia, The Free Encyclopedia. Retrieved 12:15, May 9, 2018, from https://en.wikipedia.org/w/index.php?title=Chlorophyll_fluorescence&oldid=825282258

WIKIPEDIA CONTRIBUTORS, 2018b. Plant stress measurement. In Wikipedia, The Free Encyclopedia. Retrieved 12:12, May 9, 2018, from https://en.wikipedia.org/w/index.php?title=Plant_stress_measurement&oldid=814796698

ŻUREK, G., RYBKA, K., POGRZEBA, M., KRZYŻAK, J., & PROKOPIUK, K., 2014. Chlorophyll a fluorescence in evaluation of the effect of heavy metal soil contamination on perennial grasses. PLoS One. **9.** (3) e91475.