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Phytoextraction of toxic elements and chlorophyll fluorescence in the leaves of energy willow (*Salix* sp.), treated with wastewater solids and wood ash

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Abstract

Open-field small plot long-term experiment was set up during 2011 with willow (*Salix triandra* × *S. viminalis* 'Inger'), grown as a short rotation coppice energy crop in Nyíregyháza, Hungary. The sandy loam Cambisol with neutral pH was treated three times (2011, 2013, and 2016) with 15 t ha^{-1} municipal sewage sludge compost (MSSC) and with 600 kg ha^{-1} (2011, 2013) or 300 kg ha^{-1} (2016) wood ash (WA). In 2018 the MSSC-treated plots were amended with 7.5 t ha^{-1} municipal sewage sediment (MSS), and 300 kg ha^{-1} WA. MSSC and WA or MSS and WA were also applied to the soil in combinations during all treatments. Control plots remained untreated since 2011. Repeated application of wastewater solids (MSSC, MSS) and wood ash (WA) significantly enhanced the amounts of As (up to +287%), Ba, Cd (up to +192%), Cu, Mn, Pb, and Zn in the topsoil of willows. The combined application of MSSC+MSS+WA resulted in significantly higher Mn and Zn and lower As Ba, Cd Cr, and Pb concentrations in topsoil than MSSC+MSS treatment of soil without WA. Nitrogen concentrations in leaves of treated plants were generally slightly lower or similar to control. All soil treatments significantly enhanced the uptake or accumulation of nutrient elements (Ca, K, Mg, P) and potentially toxic elements (As, Ba, Cd, Cr, Cu, Mn, Ni, Pb, and Zn) in the leaves of willows during 2018, 2019, and 2020. Significantly higher Mn or Zn concentrations were measured in MSSC+MSS+WA than in MSSC+MSS treatments. Significant amounts of Cd (up to 1.11 mg kg^{-1}) or Zn (up to 183 mg kg^{-1}) can be translocated (phytoextracted) from a soil amended with wastewater solids or wood ash to willow leaves. In 2018 the treatments decreased the chlorophyll fluorescence values, while in 2019 and 2020 the light adapted fluorescence yield (Y) values were higher in treated than in control plants.

Keywords: phytoextraction, photosynthesis, energy willow (*Salix* sp.), wastewater solid, wood ash

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Introduction

Steadily increasing emission of carbon dioxide concentration in the air associated with burning of fossil fuels, and danger of global warming focused attention on energy production from biomass. *Bioenergy*, in the form of cellulosic biomass, starch, sugar, and oils from crop plants, has emerged as one of the cheaper, cleaner, and environmentally sustainable alternatives to traditional forms of energy. Burning of the biomass of so-called *energy crops* can significantly mitigate anthropogenic emission of carbon dioxide, and can partially replace fossil fuels (STREZOV & ANAWAR, 2019; SIMON et al., 2022). Those herbaceous (e.g., *Agropyron*, *Arundo*, *Sida*, *Panicum*, *Miscanthus* spp.) or woody (e.g., *Populus*, *Robinia*, *Salix*, *Paulownia* spp.) plant species are considered as energy crops that are primarily cultivated in plantations for biomass production and energetical utilisation (KOLE et al., 2012; SIMON et al., 2022).

Rapidly growing willow (*Salix*) species cultivated for their high aboveground biomass are very promising energy crops all over the world. Harvestable shoot wet biomass of willows can achieve up to 10–12 tons of dry matter (d.m.) per hectare annually (MERILO et al., 2006; KULIG et al., 2019). The willow sprouts well, so its 2–6-meter-long shoots can be harvested on an annual, biennial, or triennial basis (GYURICZA et al., 2008; SMART & CAMERON, 2012; SIMON et al., 2016).

Since *short-rotation coppice* (SRC) energy plantations can be cultivated for 15–20 years in the same field, balanced and regular nutrient supply is required in the soil to obtain good aboveground biomass yields (GYURICZA et al., 2008; SMART & CAMERON, 2012). Biomass yield of *Salix* spp., grown as an energy crop, can be stimulated by the application of various inorganic or organic fertilizers and additives in soil, including biosolids (e.g., municipal sewage sludge, compost), biochar or biomass ash (PULFORD et al., 2002; PARK et al., 2005; DIMITRIOU et al., 2006; SALETNIK & PUCHALSKI, 2019). Since municipal sewage sludge is not a balanced fertiliser in terms of plant nutrients (it contains mainly P and organically bound N, but very low amount of K), it could be advantegeous to mix it with wood ash that contains K and some P (DIMITRIOU et al., 2006; JAMA & NOWAK, 2012). It is well documented that willow plants react well for balanced fertilization, and the enhanced uptake of nitrogen in leaves or shoots is increasing the harvestable shoot yield (WEIH & RÖNNBERG-WÄSTLJUNG, 2007; SIMON et al., 2016; SIMON et al., 2018; MERILO et al., 2006). However, application of various soil amendments (e.g., municipal sewage sludge or wood ash) can enhance not only the uptake rate of beneficial elements (e.g., nitrogen or potassium), but also the accumulation rate of *potentially toxic elements* (PTEs; e.g. arsenic – As, cadmium – Cd, or zinc – Zn) in the willow organs (PARK et al., 2005; DIMITRIOU et al., 2006; GYURICZA et al., 2008; MAXTED et al., 2007; JAMA & NOWAK, 2012; SIMON et al., 2016; SALAM et al., 2019; LABRECQUE et al., 2020; SALETNIK et al., 2020; WÓJCIK et al., 2020). This may have an impact on the toxic metal concentration of harvested shoots and thus also on the toxic metal concentration of ash after biomass burning.

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Phytoextraction is the use of plants for the removal of inorganic contaminants from the contaminated matrix through their uptake into the harvestable parts of the plant. Several factors contribute to the success of phytoextraction as a remediation technology including the extent of contamination, metal bioavailability, and the plant's ability to intercept, absorb, and accumulate metals (ARTHUR et al., 2005; SIMON, 2004). One of the basic strategies for phytoextraction of PTEs from contaminated soil is cultivating fast-growing plant species with high biomass production (SAAMENA & PUTHUR, 2021). Bioenergy plants (including *Salix* spp.) have the potential to adapt well in the polluted lands and have the capacity to produce high biomass along with high energy potential (JHA et al., 2017). In the majority of cases by bioenergy plants, heavy metal concentrations found in the shoot biomass usually remain below the standard toxicity levels. Therefore, the shoot biomass of bioenergy plants utilized for phytoremediation can be safely used for bioenergy production (JHA et al., 2017; SAMEENA & PUTHUR, 2021).

It is well documented, that compared to other plant species and to other trace elements Cd accumulation or Zn uptake rates of *Salix* spp. are high (VYSLOUŽILOVÁ et al., 2003; DICKINSON & PULFORD, 2005; MAXTED et al., 2007; SIMON et al., 2018). Resistance or tolerance of willows to other metals (Cr, Cu, Ni, Pb) and accumulation of elevated levels of As, Cu, Fe, Ni, Mn and Pb (PULFORD et al., 2002; GYURICZA et al., 2008; MLECZEK et al., 2013; VANDECASTEELE et al., 2015; TŐZSÉR et al., 2018; LABRECQUE et al., 2020) was also observed in the organs of various *Salix* spp. Therefore, from the point of view of phytoextraction (phytoremediation), the elevated level of certain toxic elements could be advantageous in the shoots of SRC willow to remove metal pollutants from the soil (GREGER & LANDBERG, 1999; PULFORD & WATSON, 2003). According to MAXTED et al. (2007) *Salix*-based phytoextraction may be applied to arable soils which require only minor 'polishing' in order to meet arable soil standards or produce metal concentrations in edible crops which do not violate risk-based hazard quotients.

Considering the above preliminaries our aim was to investigate the uptake of 5 nutrient elements (N, P, K, Ca and Mg) and the accumulation of 9 selected PTEs (As, Ba, Cd, Cr, Cu, Mn, Ni, Pb and Zn) in leaves of energy willow grown in a long-term experiment. It was assumed that repeated soil application of wastewater solids (municipal sewage sludge compost – MSSC, municipal sewage sediment – MSS) or wood ash (WA) will influence the uptake of nutrient elements, or the accumulation of PTEs in willow leaves differently. It was presumed that changed uptake of nutrient elements or PTEs impact the chlorophyll fluorescence and thereby photosynthesis rate in the willow leaves. Special attention was paid to the repeated WA soil application in combination with MSSC or MSS, and its long-term impact on PTEs' accumulation in willow, since relatively few investigations (DIMITRIOU et al., 2006; ADLER et al., 2008; LAZDINA et al., 2011) focused on this formerly. WA was supposed to reduce the accumulation of PTEs in willow leaves when applied altogether with MSSC and MSS.

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Materials and Methods

Long-term open-field experiment with willow, soil treatments

Open-field small plot long-term experiment was set up with energy willow (*Salix triandra* × *Salix viminalis* 'Inger') during 2011. Research area is located in parallel to Westsik street in Nyíregyháza (Hungary; geographical coordinates: 47°58'41.8"N 21°42'00.7"E) in the experimental field of University of Debrecen, IAREF, Research Institute of Nyíregyháza. Total area of the long-term experiment is 3,800 m^2. The experiment was set up on random-block design on 40 small plots with 10 various treatments, and with 4 replications.

Period of soil treatments was between April 2011 and June 2018, and period of presented soil and plant observations was from June 2018 to September 2020.

Willows were planted during April 2011, cuttings originated from Holland-Alma Ltd., Piricse, Hungary (license holder of the studied willow species is Lantmännen Agroenergi AB, Sweden). In each 27 m^2 experimental plot 40 willow bushes were grown with 0.75 m line spacing and 0.6 m in between plants. In every small plot, plants were grown in two twin rows. Between twin rows, the line spacing was 1.5 meters.

Before soil treatments (during April 2011) the basic characteristics (SIMON et al., 2018) of the uncontaminated Cambisol (brown forest soil with clay stripes) were the following at 0–25 cm depth: loamy sand texture; pH-H_2O: 8.10; pH-KCl: 7.52; total salt (m m^{-1} %): <0.02; $CaCO_3$ (m m^{-1} %): 4.80; humus (m m^{-1} %): 1.51%; CEC ($cmol_c$ kg^{-1}): 10.4 P–621, K–2,918, Ca–16,307, Mg–4,603; As–9.60, Ba–57.5, Cd–0.21, Cr–13.7, Cu–9.18, Mn–372, Ni–14.0, Pb–9.89, and Zn–35.5 mg kg^{-1}; as determined from cc. HNO_3–cc. H_2O_2 extract, followed the instructions of the Hungarian Standard MSZ 21470-50 (2006).

The top 0–25 cm layer of the soil was treated with municipal green waste compost, municipal sewage sludge compost, willow ash, rhyolite tuff, and fertilizers (ammonium nitrate, urea, urea with sulphur) as top-dressings during April, May, or June of 2011, 2013, and 2016 (*Figure 1*; SIMON et al., 2016, 2018).

Municipal sewage sludge compost (MSSC; producer Nyírségvíz Ltd., Nyíregyháza, Hungary) was applied to the top layer of the soil 3 times (during June 2011, May 2013, and May 2016) in 15 t ha^{-1} (wet weight with 48–56% d.m.) dose each year. PTEs' concentrations during 2016 in cc. HNO_3–cc. H_2O_2 extract (MSZ 21470-50, 2006) are shown in *Table 1*. Other basic physical and chemical characteristics and plant nutrient content of MSSC (SIMON et al., 2018) were the following: pH-H_2O 5.93; pH-KCl 5.91, $CaCO_3$ (m m^{-1} %): 0; total salt content (m m^{-1} %): 3.34; total C (m m^{-1} %): 10.4; total N (m m^{-1} %): 1.84; NH_4-N (mg kg^{-1}): 169; NO_3-N (mg kg^{-1}): 42.3; P–18,876, K–3,424, Ca–39,294, Mg–4,479, Fe–17,149 mg kg^{-1} in cc. HNO_3–cc. H_2O_2 extract (MSZ 21470-50, 2006).

Wood ash (WA) was prepared with burning of leafless twigs of the willows, grown formerly in the experimental plots. Topsoil of the plots was treated three times with WA, in June 2011 and May 2013 with 600 kg ha^{-1} doses, respectively; and in May 2016 with 300 kg ha^{-1} dose. PTE concentrations of WA (99% d.m.) during 2016

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are presented in *Table 1*. Applied WA can be defined with the following basic characteristics (SIMON et al., 2018): pH-H_2O 10.9; pH-KCl 10.7, total salt content (m m^{-1} %): 1.17; NH_4-N (mg kg^{-1}):0; NO_3-N (mg kg^{-1}):0; P–6,472, K–16,508, Ca–43,074, Mg–7,991, Fe–17,045 mg kg^{-1} in cc. HNO_3–cc. H_2O_2 extract (MSZ 21470-50, 2006).

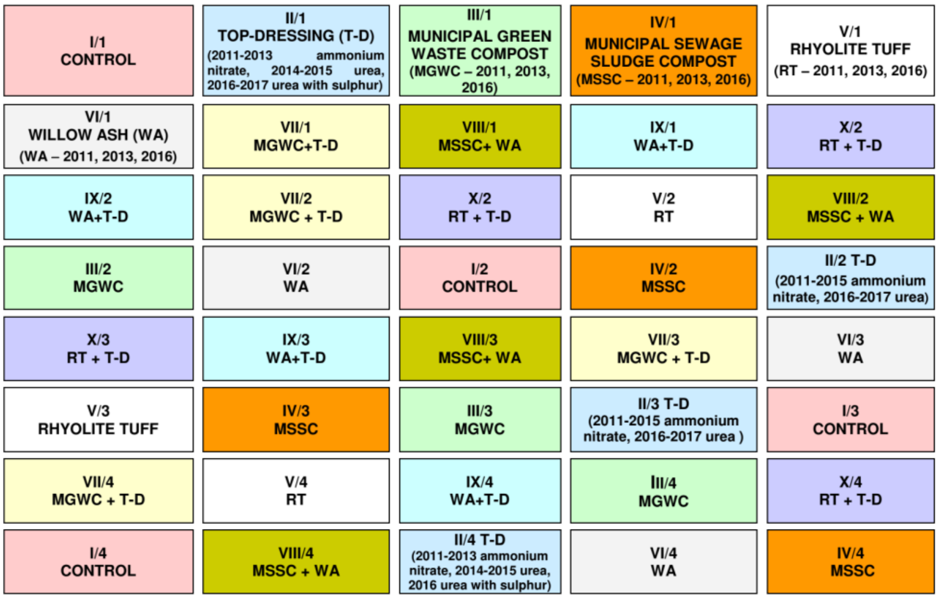

Figure 1
Scheme of the long-term experiment with energy willow (Salix triandra × Salix viminalis 'Inger'), soil treatments between 2011–2017 in random block layout with 4 replications (Nyíregyháza, Hungary)

Above amendments and fertilizers (immediately rotated to upper 0–25 cm layer of the soil) were applied to the soil between 2011 and 2017 years also in various combinations (e.g. 15 t ha^{-1} MSSC+300 kg ha^{-1} WA during 2016, see SIMON et al., 2016, 2018). Control plots remained untreated (unfertilised) throughout the experiment (*Figure 1*).

During March and April 2018, the shoots of all willow bushes were harvested (this was the 3rd harvest after 2013 and 2016).

On 15 June 2018 the soil of plots formerly (2011, 2013, 2016) treated 3 times with MSSC were amended with air dry, unscreened 7.5 t ha^{-1} dose of *municipal sewage sediment* (MSS) in 4 replications (*Figure 2*; SIMON et al., 2022). MSS originated from Lovász-zug suburban area of Debrecen, Hungary (47°29′07″ N, 21°35′46″ E), where formerly a sewage settling pond was operated as a secondary biological purification unit (TŐZSÉR et al., 2018). MSS samples were collected from this recultivated sewage settling pond, where MSS was located under artificial soil

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cover in a 70–110 cm depth. Approximately 280–320 kg wet MSS samples were then spread in a 10–15 cm layer, regularly rotated, shredded, and air-dried in a covered, aerated building of the University of Nyíregyháza (Hungary) for 2 months. Four composite samples were taken from the air dry MSS for chemical analysis. One composite sample with 1.0–1.5 kg total mass arises from combining 25 subsamples. Thoroughly mixed composite samples were passed through a 5-mm diameter sieve before analysis. *Table 1* shows the concentrations of PTEs in this substance.

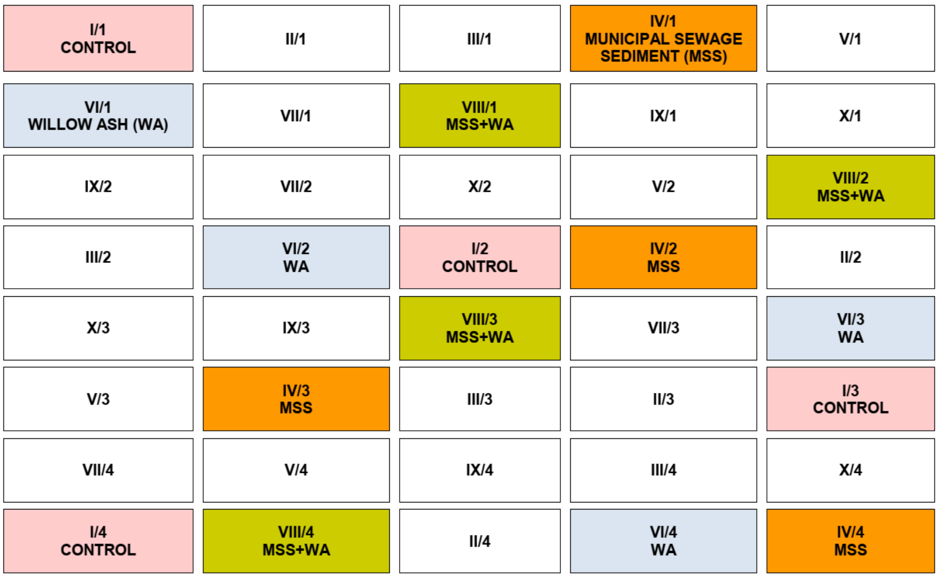

Figure 2
Scheme of the long-term experiment with energy willow (Salix triandra × Salix viminalis 'Inger'), soil treatments during 2018 (Nyíregyháza, Hungary)

Wood ash was prepared at the beginning of June 2018 with burning of dry leafless twigs of the willows from the 2016 harvest. WA was passed through 8-mm sieve before its soil application, and sampled for chemical analysis, as described above for MSS. On 15 June 2018 the soil of plots formerly (2011, 2013, 2016) treated 3 times with WA was again amended with a completely dry, 300 kg ha^{-1} dose of willow ash in 4 replications (*Figure 2*; SIMON et al., 2022). Control plots remained untreated. MSS and WA were also applied to the soil in combinations during 2018 treatments. PTE concentrations of WA during 2018 are presented in *Table 1*.

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Table 1
"Pseudo-total" concentrations of potentially toxic elements in the soil additives applied to the topsoil of energy willow (Salix triandra × Salix viminalis 'Inger'), (Nyíregyháza, Hungary)*

PTE ($mg\ kg^{-1}$)	Soil additives (year of application)			
	MSSC (2016)	**WA (2016)**	**MSS (2018)**	**WA (2018)**
As	12.2	10.2	31.0	18.5
Ba	212	267	596	403
Cd	0.55	2.38	1.23	0.60
Cr	19.3	9.66	1142	9.10
Cu	79.0	133	198	130
Mn	318	553	520	670
Ni	15.1	10.9	62.8	14.2
Pb	22.0	12.1	278	26.7
Zn	357	1757	978	1853

**cc. HNO_3 + cc. H_2O_2 extract. Data are means of 4 replications.*

Soil sampling

To check the impacts of the 3 times (2011, 2013, 2016) applied MSSC, WA, or MSSC+WA on the concentrations of PTEs in soil, samples were taken on the 7th of June 2018 from 16 plots, including controls. Approximately 1.2–1.5 kg composite soil samples per experimental plot were collected, drilling 10 cm far from the stems of 25 willow bushes. Twenty five subsamples per plot were taken from 0–25 cm depth using a standard gouge auger (Eikelkamp, The Netherlands). All 16 plots included in the experiment were sampled in this method. Soil sampling was repeated by 25 September 2020 from 0–30 cm depth, as described above. All soil samplings were done in 4 replicates per treatments.

Immediately after sampling all soil samples were taken to the laboratory. After the removal of foreign substances, the soil was homogenised and spread on plastic plates in a thin layer. After 14 days of drying at room temperature the thoroughly mixed air dry samples were passed through 2-mm sieve.

Plant sampling

First sampling of willow leaves was done 5 weeks after application of MSS, WA and MSS+WA soil amendments, on 25 July 2018. Willow leaves were sampled from 10 plants per plot. Five individuals of the sampled plants were from the middle section of the 2nd row, while five were from the middle section of the 3rd row of a given plot. Ten fully developed leaves per plant were collected from the10−20 cm uppermost section of the shoots. From each plot 100 leaves, from each treatment 400 leaves were collected, with an avarege total fresh weight of 79-gram per plot. The second sampling of willow leaves was conducted on 24 June 2019; 53 weeks after the last soil treatments. The sampling method was identical that in the previous year, in average 46-gram leaves were collected per plot. The third willow leaf sampling was performed 101 weeks (on 27 May 2020) after the last soil treatments. The sampling protocol was the same as in 2018 and 2019. In 2020, the average fresh

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weight of the 100 collected willow leaves per experimental plot was 35 g. All 16 plots included in the experiment were sampled similarly. Plant sampling was done in 4 replicates per treatments.

Immediately after sampling, leaves were thoroughly washed in flowing tap water in the laboratory. The tap water was rinsed from the samples in two-times-changed monodistilled water. Leaf samples were dried until constant loss of weight in drying oven (Mytron, Germany) at 70^0C for 10 hours. Dry samples were ground to particles <1 mm in an ultracentrifugal mill (Retsch ZEM 200, Germany).

Leaf chlorophyll fluorescence measurements

Leaf chlorophyll fluorescence measurements were conducted on 25 July 2018, 25 June 2019, and 30 July 2020 with an OS5p type (Opti-Sciences, Inc., USA) chlorophyll fluorescence meter, which measures the quantum yield, after emission of excitation light on the leaf surface. Based on the amount of energy emitted by the excitation light, the amount of quantum utilization (Yield, Y) of the PS-II photocenter was measured. This value generally correlates well with the degree of carbon assimilation (CAVENDER-BARES & BAZZAZ, 2004). Measurements were performed on light-adapted plants, in each case in the morning when there was no cloudy sky.

Measurements were done in fully developed leaves, which were located at the 30–60 cm uppermost section of the shoots. At each experimental plot, 10 bushes (positioned on the 2^{nd} or 3^{rd} rows) were chosen from the middle section of the parcels, where the measurements were conducted on the top 4^{th} (during 2018–2019), top 8^{th}, 9^{th} and 10^{th} positioned (2020) healthy leaves.

Elemental analysis of soil, soil additive, and plant samples

To determine the “pseudo-total” element content of the *soil or soil additives*, the Hungarian Standard MSZ 21470-50 (2006) was followed with a slight modification. From the prepared (dried and ground to particles <0.1 mm) soil and soil additive samples, 0.5 g was loaded into the pressure-proof bombs of the microwave digester (Milestone Ethos Plus, Italy). To all samples, 6 ml of distilled cc. HNO_3 and 2 ml 30% (v v^{-1}) H_2O_2 was added. For soil samples or soil additives the digestion was performed by the Application Note 031 programme of the microwave digester, as follows; 10 min to 200 °C followed by 15 min at 200 °C. Digested samples were washed into a 50 ml volumetric flask with distilled water, homogenized and filtered (MN 640 W paper; Macherey-Nagel, Germany).

From the prepared (dried and ground to particles <0.1 mm) *plant samples*, 0.5 g was loaded into the pressure-proof bombs of the microwave digester. To all samples, 5 ml of distilled cc. HNO_3 and 3 ml 30% (v v^{-1}) H_2O_2 (Scharlau, Spain) was added. The digestion was performed by the Application Note 076 programme of the microwave digester, as follows; 3 min to 85 °C, 9 min to 145°C; 4 min to 200°C; 14 min at 200°C.

Elemental analysis of all samples was conducted with Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) technique, applied on iCAP 7000 spectrophotometer (ThermoFischer Scientific, USA). For the calibration, a multielement standard solution (n = 2) was applied. Nitrogen concentration of plant

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samples was determined by Kjeldahl method. All elemental analyses were done with 4 replicates.

Statistical analysis

Statistical analysis of experimental data was conducted with SPSS 26.0 software using analysis of a variance (ANOVA) followed by treatment comparison using Tukey's b-test. The relationships between leaf parameters were studied with Pearson's correlation.

Results and Discussion

Elemental composition of soil

It was assumed that surpluses of PTEs (*Table 1*) from three times applied MSSC and WA would be measurable in the topsoil of the experimental plots. To prove this, soil samples were collected in June 2018 from the experimental plots. *Table 2* presents the concentrations of 9 selected PTEs in the upper layer of the experimental soils.

All treatments significantly ($P < 0.05$) enhanced the concentrations of all PTEs in soils, as compared to the control. The only exception was Cr in WA-treated soil (*Table 2*). The highest As, Ba, Cd, Cu, Cr, Ni, and Pb concentrations were detected in MSSC-treated soil, while the most elevated Mn and Zn contents were present in WA-treated soil. This was related to significant amounts of Mn and Zn present in willow ash (*Table 1*). Except for Mn and Zn, the co-application of MSSC and WA resulted in significantly lower PTEs concentrations in soil than the application of MSSC alone.

Our results confirm the well-known phenomenon that single or repeated application, long-term soil disposal of municipal sewage sludge or wood ash can considerably enhance the concentrations of PTEs in topsoil (PULFORD et al., 2002; DIMITRIOU et al., 2006; MAXTED et al., 2007). Except As, PTEs concentrations measured in soils of our experimental plots were, however, equally lower than the valid Hungarian threshold limits (As–15, Ba–250, Cd–1, Cr–75, Cu–75, Ni–40, Pb–100, and Zn–200 mg kg^{-1}) for soil pollution (KVVM-EÜM-FVM, 2009).

On 15 June 2018 the soil of plots formerly treated 3 times with MSSC was amended with MSS. Chemical analysis of MSS revealed that this wastewater solid is rich in calcium (Ca; 34,724 mg kg^{-1} d.m.), magnesium (Mg; 7,049 mg kg^{-1} d.m.), phosphorus (P; 4,695 mg kg^{-1} d.m.), and potassium (K; 3,077 mg kg^{-1} d.m.), (SIMON et al., 2022). The WA applied at the same time, however, contained 17 times more K (54,248 mg kg^{-1} d.m), or appr. 5 times more Ca (187,550 mg kg^{-1} d.m.), Mg (35,348 mg kg^{-1} d.m) and P (25,403 mg kg^{-1} d.m.) than MSS (SIMON et al., 2022). These values are in agreement with observations of other authors (PARK et al., 2005; DIMITRIOU et al., 2006; LAZDINA et al., 2011; SALETNIK & PUCHALSKI, 2019; WÓJCIK et al., 2020), who noticed that sewage sludge or wood ash is a rich source of Ca, Mg, P or K. The MSS contained significantly more Cr and Pb than WA, while WA contained more Zn (*Table 1*). The concentrations of PTEs in WA are in the range

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observed by ZAJĄC et al. (2019), SALETNIK & PUCHALSKI (2019) and WÓJCIK et al. (2020) in willow ash or wood ash.

Table 2

"Pseudo-total" concentrations of potentially toxic elements in the topsoil of the open-field long-term experiment set up with energy willows (Salix triandra × Salix viminalis 'Inger'), (Nyíregyháza, Hungary)*

PTE ($mg\ kg^{-1}$)	**Soil treatments (2011, 2013, 2016)**			
	Control	**MSSC**	**WA**	**MSSC+WA**
	Soil depth 0–25 cm (sampling 7 June, 2018)			
As	8.45[a]	22.3[d]	13.4[b]	19.8[c]
Ba	97.8[a]	130[c]	117[b]	127[c]
Cd	0.291[a]	0.805[d]	0.470[b]	0.642[c]
Cr	9.51[a]	18.0[c]	9.58[a]	12.0[b]
Cu	11.3[a]	15.5[d]	12.4[b]	13.7[c]
Mn	263[a]	429[b]	481[d]	454[c]
Ni	11.3[a]	14.9[d]	12.6[b]	13.9[c]
Pb	15.4[a]	35.3[d]	16.6[b]	23.1[c]
Zn	40.3[a]	51.4[b]	55.6[d]	54.0[c]
	Soil treatment (2018)			
	Control	**MSS**	**WA**	**MSS+WA**
	Soil depth 0–30 cm (sampling 24 September, 2020)			
As	9.19[a]	35.6[d]	27.3[b]	30.4[c]
Ba	56.0[a]	67.3[d]	59.6[b]	63.5[c]
Cd	0.214[a]	0.625[d]	0.391[b]	0.494[c]
Cr	12.6[a]	17.5[c]	13.1[a]	15.8[b]
Cu	9.47[a]	12.0[d]	10.1[b]	11.2[c]
Mn	368[a]	482[b]	529[b]	500[b]
Ni	13.5[a]	14.1[b]	14.9[c]	14.7[c]
Pb	10.1[a]	13.7[d]	11.4[b]	12.4[c]
Zn	35.3[a]	40.2[b]	43.2[b]	41.9[b]

**cc. HNO_3 + cc. H_2O_2 extract. Data are means of 4 replications. ANOVA Tukey's b-test. Means within the rows followed by the same letter are not statistically different at $P < 0.05$.*

In Hungary the 50/2001 GOVERNMENT DECREE (2001) controls the agricultural utilization of sewage sludge. The Cr content of the studied MSS exceeded the 1000 $mg\ kg^{-1}$ limit value for total chromium in sewage sludge. In the 36/2006 Decree of the Ministry of Agriculture and Rural Development for various crop-enhancing substances (mineral fertilizers, inorganic soil improvers) containing waste, the limits are 10 $mg\ kg^{-1}$ for As, 2 $mg\ kg^{-1}$ for Cd, 100 $mg\ kg^{-1}$ for Cr, 100 $mg\ kg^{-1}$ for Cu, 50 $mg\ kg^{-1}$ for Ni, and 100 $mg\ kg^{-1}$ for Pb (FVM DECREE, 2006). In the studied WA, As and Cu concentrations were above these regulatory limits.

Soil sampling for PTEs concentration analysis was repeated on 24 September 2020, which was 116 weeks after the last soil treatments, done on 15 June 2018. Results are shown in *Table 2*.

The 2020 year data confirm our observations from year 2018 that all soil additives, to a varying degree but uniformly, significantly increased the "pseudo-

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total" concentrations of the studied group of PTEs in topsoil, as compared to untreated control. Comparing the data of 2018 with those of 2020, it can be also stated that the relative concentrations of As and Cd increased, while those of Ba, Cu, Mn, Ni, Pb and Zn decreased during 2020, in all treated topsoils; as related to untreated control soil. These changes can be attributed to various rate downward migration (leaching) of PTEs (toxic element excess appeared in the "pseudo-total" and "plant available" fractions of 30–60 cm soil layer; SIMON et al., 2022), to various rate of binding of PTEs to soil colloids, or to uptake or accumulation (phytoextraction) of PTEs in willow organs, including leaves (*Table 3*).

Similarly to 2018 year results (*Table 2*) it was recognized during 2020 that co-application of MSSC or MSS and WA resulted in significantly lower "pseudo-total" As, Ba, Cd, Cr, Cu, and Pb concentrations in topsoil than application of MSSC+MSS alone. In other respects, it can be again concluded that only Mn and Zn were added from willow ash in significant amounts to soils treated with MSSC+MSS+WA, since the Mn and Zn concentrations in MSSC+MSS+WA were higher than in MSSC+MSS soil treatments.

During September 2020 in the 0–30 cm layer of MSSC+MSS, WA or MSSC+MSS+WA treated soils 197–287% more As, 6–20% more Ba, 83–192% more Cd, 4–39% more Cr, 7–27% more Cu, 31–44% more Mn, 4–10% more Ni, 13–36% more Pb, and 14–22% more Zn was detected in HNO_3–H_2O_2 extracts (*Table 2*), as compared to PTE concentrations in untreated control soil. The most significant increment was observed in the amount of As and Cd in treated soils.

Elemental composition of willow leaves

Table 3 presents the effects of various soil treatments on the concentrations of nutrient elements (macroelements) in the leaves of energy willows. In July 2018, compared to control, it was found that the most significant increase in the concentration of P, K, Ca, and Mg in leaves can be observed if willow ash was applied to the soil. All other former soil treatments (MSSC+MSS or MSSC+MSS+WA) also enhanced significantly the P, K, Ca, or Mg concentrations in willow leaves. In the case of nitrogen concentration, in contrast to the other elements assessed, the highest value was measured in the control leaves, and slightly lower element contents were detected as a result of the different treatments. Since almost all former treatments in our long-term experiment resulted in higher harvestable wood biomass than in untreated control (SIMON et al., 2017), the decrease in releative concentration of nitrogen could be attributed to so-called 'dilution effect'. Presumably, the treated plants absorbed more nitrogen from the soil than the control, however, this was distributed, 'diluted' in the larger aboveground biomass.

During the second leaf sampling in June 2019, similar phenomena were observed (*Table 3*); with the exception of nitrogen, the highest amounts of macronutrients were found in treated plants. As a result of the treatments, there was up to 15% decrease in the concentration of N in willow leaves, while P was present at 22% higher concentration in the leaves in MSSC+MSS treated plants than in control. Highest K (+14%) or Mg (+6%) concentrations were found in WA-treated leaves. Calcium concentrations were 15% higher in MSSC+MSS+WA treatment than

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in control. Since wastewater solids are rich sources of phosphorus (JAMA & NOWAK, 2012), and wood ashes are rich in potassium (DIMITRIOU et al., 2006), it could be presumed, that the surplus of P and K in the willow leaves originated from these soil amendments.

The results of the third leaf sampling in May 2020 confirmed the results of the preceding years (*Table 3*). In terms of P and Ca, we found the highest element uptake in the leaves of MSSC+MSS treated plants. For K and Mg, however, the most significant increase in leaf concentration was found in WA treatment, compared to the untreated control.

Table 3

Effects of soil treatments on the concentrations of nutrient elements in the leaves of energy willows (Salix triandra × Salix viminalis 'Inger'), grown in a long-term open-field experiment (Nyíregyháza, Hungary)

Concentrations of nutrient elements	**Soil treatments (2011, 2013, 2016, 2018)**			
	Control	**MSSC+MSS**	**WA**	**MSSC+MSS+WA**
		Willow leaves (25 July 2018)		
N (m m^{-1} %)	2.22^{a}	2.18^{a}	2.08^{a}	2.16^{a}
P (mg kg^{-1})	3141^{a}	3341^{b}	3638^{d}	3500^{c}
K (mg kg^{-1})	5257^{a}	6117^{b}	7033^{c}	6250^{b}
Ca (mg kg^{-1})	10399^{a}	11085^{b}	14556^{d}	12981^{c}
Mg(mg kg^{-1})	5654^{a}	5860^{b}	6986^{d}	6519^{c}
		Willow leaves (24 June 2019)		
N (m m^{-1} %)	2.60^{c}	2.22^{a}	2.38^{b}	2.29^{ab}
P (mg kg^{-1})	3637^{a}	4425^{d}	3952^{b}	4133^{c}
K (mg kg^{-1})	10462^{a}	10913^{a}	11964^{b}	11487^{b}
Ca (mg kg^{-1})	8665^{a}	9962^{c}	9765^{b}	9973^{c}
Mg(mg kg^{-1})	3879^{a}	3974^{a}	4124^{b}	3991^{a}
		Willow leaves (27 May 2020)		
N (m m^{-1} %)	2.53^{a}	2.50^{a}	2.65^{a}	2.51^{a}
P (mg kg^{-1})	4425^{a}	6122^{c}	5829^{b}	6014^{bc}
K (mg kg^{-1})	11434^{a}	12229^{b}	13462^{c}	12609^{b}
Ca (mg kg^{-1})	9828^{a}	12608^{d}	10597^{b}	11656^{c}
Mg(mg kg^{-1})	4739^{a}	5658^{b}	6162^{c}	5983^{bc}

Data are means of 4 replications. ANOVA Tukey's b-test. Means within the rows followed by the same letter are not statistically different at P < 0.05.

Table 4 shows the concentrations of selected PTEs in the leaves of willows grown in soils repeatedly treated with MSSC, MSS, or WA. It can be generally declared that all former soil treatments enhanced significantly the uptake or accumulation of PTEs in the leaves of willows. Elevated levels of As, Cd, Cr, Cu, Ni, or Pb in leaves of willow are, however, in the normal range for plants (KABATA-PENDIAS, 2011; SIMON, 2014). Only Zn concentrations (160–183 mg kg^{-1} d.m.) measured during 2018 in leaves can be considered slightly excessive, considering that 100-400 mg kg^{-1} of zinc in mature leaf tissue is excessive or toxic (KABATA-PENDIAS, 2011; SIMON, 2014).

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Table 4
Effects of soil treatments on the concentrations of potentially toxic elements in the leaves of energy willows (Salix triandra × Salix viminalis 'Inger'), grown in a long-term open-field experiment (Nyíregyháza, Hungary)

PTE	Soil treatments (2011, 2013, 2016, 2018)			
(mg kg^{-1})	Control	MSSC+MSS	WA	MSSC+MSS+WA
	Willow leaves (25 July 2018)			
As	0.174^{a}	0.276^{d}	0.218^{b}	0.250^{c}
Ba	6.18^{a}	9.12^{c}	8.56^{b}	8.89^{bc}
Cd	0.806^{a}	1.111^{d}	0.974^{b}	1.041^{c}
Cr	0.231^{a}	0.473^{d}	0.323^{b}	0.399^{c}
Cu	8.71^{a}	14.8^{c}	9.08^{a}	10.9^{b}
Mn	44.6^{a}	51.0^{b}	71.2^{d}	60.8^{c}
Ni	1.08^{a}	2.28^{d}	1.76^{b}	2.03^{c}
Pb	0.156^{a}	0.568^{d}	0.180^{b}	0.372^{c}
Zn	123^{a}	160^{b}	183^{d}	173^{c}
	Willow leaves (24 June 2019)			
As	0.176^{a}	0.295^{d}	0.200^{b}	0.243^{c}
Ba	4.66^{b}	4.22^{a}	6.89^{d}	5.47^{c}
Cd	0.847^{b}	0.913^{d}	0.792^{a}	0.883^{c}
Cr	0.266^{a}	0.543^{d}	0.348^{b}	0.461^{c}
Cu	8.04^{a}	8.99^{c}	8.40^{b}	8.54^{b}
Mn	35.8^{a}	34.3^{a}	41.1^{c}	37.9^{b}
Ni	1.06^{b}	1.57^{d}	0.97^{c}	1.21^{c}
Pb	0.097^{a}	0.308^{c}	0.114^{a}	0.220^{b}
Zn	79.7^{c}	67.9^{a}	79.9^{c}	73.0^{b}
	Willow leaves (27 May 2020)			
As	0.194^{a}	0.439^{c}	0.349^{b}	0.392^{bc}
Ba	5.45^{a}	5.83^{a}	6.46^{b}	6.34^{b}
Cd	0.872^{b}	0.994^{c}	0.768^{a}	0.868^{b}
Cr	0.304^{a}	0.683^{d}	0.395^{b}	0.516^{c}
Cu	8.13^{a}	9.39^{d}	8.66^{b}	9.01^{c}
Mn	39.0^{a}	50.5^{b}	56.2^{c}	53.7^{c}
Ni	1.02^{b}	1.33^{c}	0.93^{a}	1.06^{b}
Pb	0.110^{a}	0.874^{d}	0.332^{b}	0.587^{c}
Zn	98.5^{a}	133^{c}	109^{b}	117^{b}

Data are means of 4 replications. ANOVA Tukey's b-test. Means within the rows followed by the same letter are not statistically different at P < 0.05.

Comparing the concentrations of various PTEs in the leaves of willows during 2018, 2019, or 2020 year, it can be observed, that the overall accumulation rate was higher immediately after soil treatments during 2018 than in 2019 or 2020. The highest Ba, Cd, Cu, Mn, Ni, and Zn concentrations were measured in the leaves 5 weeks after soil treatments during 2018. Later, during 2019 or 2020, 53 or 101 weeks after last soil treatments the accumulation rate of PTEs in leaves decreased (*Table 4*). During May 2020, however, in leaves of MSSC+MSS-, WA- or MSSC+MSS+WA-treated willows still 78–124% more As, 7–18% more Ba, 30–125% more Cr, 7–15%

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more Cu, 29–44% more Mn, 202–695% more Pb, or 11–35% more Zn was detected, than in untreated control. During 2020, only MSSC+MSS treatments enhanced significantly the Cd accumulation in leaves by 14%, while former WA treatments reduced it by 12%, as compared to control (*Table 4*). This is in agreement with our observation that during 2020 the "pseudo-total" Cd concentrations in treated topsoils were lower than in 2018 (*Table 2*). During 2020, besides 'pseudo-total' 'plant available' concentrations of Cd were also higher in MSSC+MSS than in WA-treated soil (SIMON et al., 2022).

It was observed in soils that MSSC+MSS+WA treatments resulted in more "pseudo-total" Mn and Zn in topsoil than MSSC+MSS application by itself (*Table 2*). This was reflected in leaves of willows, where significantly higher Mn or Zn concentrations were measured in MSSC+MSS+WA than in MSSC+MSS treatments during all observation periods (*Table 4*).

Bioconcentration factors (BCF; ratio of a PTE concentration in the willow leaves to PTE "pseudo-total" concentration in soil) were calculated according to BUSCAROLI (2017) to estimate a plant's potential for phytoremediation, phytoextraction purposes. On the basis of 2020 year' values of accumulated PTEs in leaves of willows (*Table 4*) and of "pseudo-total" concentrations of PTEs in soils (*Table 2*) the BCFs were in this order: Zn>Cd>Cu>Mn>Ba>Ni>Pb>Cr>As (SIMON et al., 2022). This is in agreement with observations of other authors (VYSLOUŽILOVÁ et al., 2003; DICKINSON & PULFORD, 2005; MAXTED et al., 2007; MLECZEK et al., 2013) that willow leaves are effective phytoextractors of zinc, cadmium and partially copper from contaminated soils.

Chlorophyll fluorescence of willow leaves

Light adapted Fluorescence Yield (Y) was measured in 3 consecutive years after the soil treatment. Generally, the Y values were higher year by year which indicates the increasing photosynthetic intensity of plants and decreasing inhibitory effects of PTEs accumulated in willow leaves (*Figure 3*). Within years, there was no treatment effect in the first year of measurements but the highest Y values were measured in control plants indicating the slightly negative effects of tested soil additives on plants assimilation processes. In 2019, the Fluorescence Yield of control plants was similar to the value measured in 2018, while the treatments resulted in 5.2%, 8.3%, and 9.8% higher Y values compared to the results of 2018. The highest value was measured in the MSSC+MSS+WA treatment, which was significantly higher than the control. In 2020, the lowest Fluorescence Yield values measured in the MSSC+MSS treatment were not significantly different from control and MSSC+MSS+WA treatments, however, the assimilation indicator was statistically higher in WA than in MSSC+MSS treatment.

According to ŻUREK et al. (2014) chlorophyll *a* fluorescence gives information about the plant physiological status due to its coupling to the photosynthetic electron transfer chain and to the further biochemical processes. While fluorescence yield can be used for getting information about plant ability to tolerate environmental stresses (including an excess of PTEs in plant leaves) (MAXWELL & JOHNSON, 2000) we can state that plant conditions were better during 2020 and 2019 than in 2018, both in

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control and treated cultures. Various PTEs could have positive or negative effects on assimilation processes in willow (KORZENIOWSKA & STANISLAWKA-GLUBIAK, 2019; URBANIAK et al., 2017; STANISŁAWSKA-GLUBIAK et al., 2012). In 2018, after the treatment of soil with materials containing toxic elements and beneficial nutrients, not significant correlations were found between Photosynthetic Yield and element content, however, all the correlations were negative indicating the unfavourable effects of studied materials on plant assimilation processes right after their soil application (*Table 5*). It is advantageous therefore that soil treatments in our experiment only slightly reduced the chlorophyll fluorescence in the leaves of willows during 2018 (*Figure 3*), in spite of the enhanced levels (*Table 4*) of PTEs in leaves. During 2019 and 2020 however, higher Y values were observed in treated than in control plants (*Figure 3*). All this can be related to the fact that the macroelement (Ca, Mg, P, K) content (*Table 3*) in willow leaves increased due to soil treatments, which can alleviate the direct toxicity of PTEs. It was indicated by the positive, however not significant correlations between Y and macroelements (*Tables 6 and 7*).

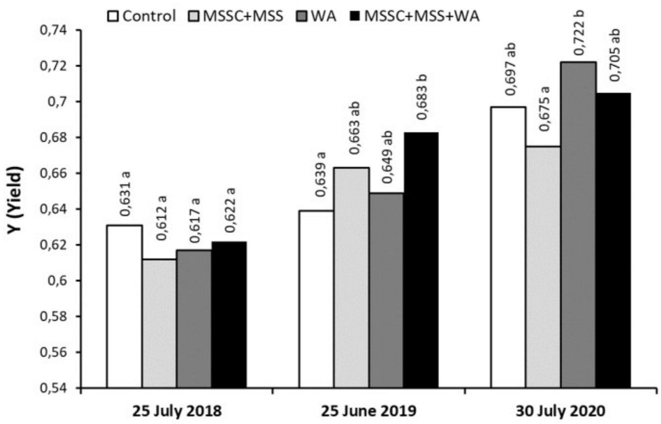

Figure 3

Effects of soil treatments on the chlorophyll fluorescence yield (Y) in the leaves of energy willows (Salix triandra × Salix viminalis 'Inger'), grown in a long-term open-field experiment (Nyíregyháza, Hungary). Data are means of 4 replications. ANOVA Tukey's b-test. Means within the columns followed by the same letter are not statistically different at $P < 0.05$.

2019 seems to be an exceptional year because the leaf N content was in negative while most of the PTEs were in weak positive correlations with Y (*Table 6*). In this year, the N concentration of treated plant leaves was lower than in control leaves (*Table 3*) explaining a negative correlation with Y. The applied soil amendments were very complex regarding their organic matter, macro-, microelements and PTEs content combined with yearly changing environmental parameters, resulting in changes in plant reactions to the treatments.

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Table 5

Correlations of elemental concentrations (25 July 2018) and chlorophyll fluorescence yield (Y) (25 July 2018) of energy willow (Salix triandra x Salix viminalis 'Inger') leaves in the open-field long-term experiment (Nyíregyháza, Hungary)

	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn	As	Ba	Cd	Cr	Ni	Pb	Yield
N	1	-0.256	-0.259	-0.314	-0.318	0.093	-0.182	-0.307	-0.245	0.013	-0.113	-0.098	-0.021	-0.055	0.096	0.026
P	-0.256	1	0.959**	0.956**	0.954**	-0.054	0.556*	0.972**	0.963**	0.382	0.698**	0.490	0.323	0.513*	-0.002	-0.230
K	-0.259	0.959**	1	0.904**	0.895**	0.038	0.580*	0.939**	0.938**	0.411	0.711**	0.529*	0.361	0.540*	0.048	-0.181
Ca	-0.314	0.956**	0.904**	1	0.993**	-0.298	0.347	0.983**	0.874**	0.161	0.509*	0.263	0.071	0.281	-0.249	-0.181
Mg	-0.318	0.954**	0.895**	0.993**	1	-0.322	0.328	0.985**	0.869**	0.137	0.492	0.249	0.059	0.266	-0.268	-0.133
Cu	0.093	-0.054	0.038	-0.298	-0.322	1	0.769**	-0.223	0.168	0.851**	0.644**	0.818**	0.901**	0.808**	0.983**	-0.239
Fe	-0.182	0.0556*	0.580*	0.347	0.328	0.769**	1	0.398	0.726**	0.941**	0.946**	0.974**	0.932**	0.980**	0.804**	-0.345
Mn	-0.307	0.972**	0.939**	0.983**	0.985**	-0.223	0.398	1	0.903**	0.197	0.571*	0.337	0.155	0.344	-0.181	-0.158
Zn	-0.245	0.963**	0.938**	0.874**	0.869**	0.168	0.726**	0.903**	1	0.570*	0.842**	0.676**	0.519*	0.695**	0.219	-0.238
As	0.013	0.382	0.411	0.161	0.137	0.851**	0.941**	0.197	0.570*	1	0.887**	0.943**	0.944**	0.954**	0.888**	-0.326
Ba	0.017	-0.237	0.808**	0.238	0.684**	-0.276	-0.768**	0.944**	0.584*	-0.434	1	-0.769**	-0.335	-0.708**	-0.522*	0.018
Cd	-0.098	0.490	0.529*	0.263	0.249	0.818**	0.974**	0.337	0.676**	0.943**	0.947**	1	0.964**	0.991**	0.855**	-0.239
Cr	-0.021	0.323	0.361	0.071	0.059	0.901**	0.932**	0.155	0.519*	0.944**	0.875**	0.964**	1	0.954**	0.929**	-0.307
Ni	-0.055	0.513*	0.540*	0.281	0.266	0.808**	0.980**	0.344	0.695**	0.954**	0.944**	0.991**	0.954**	1	0.845**	-0.263
Pb	0.096	-0.002	0.048	-0.249	-0.268	0.983**	0.804**	-0.181	0.219	0.888**	0.687**	0.855**	0.929**	0.845**	1	-0.248
Yield	0.026	-0.230	-0.181	-0.181	-0.133	-0.239	-0.345	-0.158	-0.238	-0.326	-0.268	-0.239	-0.307	-0.263	-0.248	1

*** Correlation is significant at the $P < 0.01$ level (2-tailed).*

** Correlation is significant at the $P < 0.05$ level (2-tailed).*

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Table 6

Correlations of elemental concentrations (24 June 2019) and chlorophyll fluorescence yield (Y) (25 June 2019) of energy willow (Salix triandra x Salix viminalis 'Inger') leaves in the open-field long-term experiment (Nyíregyháza, Hungary)

	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn	As	Ba	Cd	Cr	Ni	Pb	Yield
N	1	-0.914**	-0.311	-0.885**	-0.332	-0.813**	-0.589*	0.065	0.722**	-0.817**	0.017	-0.448	-0.859**	-0.656**	-0.801**	-0.508*
P	-0.914**	1	0.191	0.850**	0.256	0.913**	0.774**	-0.259	-0.880**	0.957**	-0.237	0.637**	0.971**	0.821**	0.934**	0.501*
K	-0.311	0.191	1	0.614*	0.830**	0.143	-0.384	0.775**	0.129	0.037	0.808**	-0.406	0.128	-0.324	-0.06	0.301
Ca	-0.885**	0.850**	0.614*	1	0.564*	0.771**	0.393	0.214	-0.624**	0.747**	0.238	0.295	0.819**	0.455	0.689**	0.584*
Mg	-0.332	0.256	0.830**	0.564*	1	0.245	-0.326	0.708**	0.108	0.09	0.684**	-0.411	0.173	-0.222	-0.046	0.171
Cu	-0.813**	0.913**	0.143	0.771**	0.245	1	0.696**	-0.242	-0.851**	0.902**	-0.276	0.543*	0.890**	0.802**	0.880**	0.312
Fe	-0.589*	0.774**	-0.384	0.393	-0.326	0.696**	1	-0.774**	-0.933**	0.878**	-0.768**	0.934**	0.820**	0.950**	0.927**	0.402
Mn	0.065	-0.259	0.775**	0.214	0.708**	-0.242	-0.774**	1	0.586*	-0.439	0.944**	-0.736**	-0.341	-0.728**	-0.531*	0.026
Zn	0.722**	-0.880**	0.129	-0.624**	0.108	-0.851**	-0.933**	0.586*	1	-0.970**	0.584*	-0.842**	-0.930**	-0.939**	-0.983**	-0.389
As	-0.817**	0.957**	0.037	0.747**	0.090	0.902**	0.878**	-0.439	-0.970**	1	-0.434	0.758**	0.968**	0.907**	0.983**	0.452
Ba	0.017	-0.237	0.808**	0.238	0.684**	-0.276	-0.768**	0.944**	0.584*	-0.434	1	-0.769**	-0.335	-0.708**	-0.522*	0.018
Cd	-0.448	0.637**	-0.406	0.295	-0.411	0.543*	0.934**	-0.736**	-0.842**	0.758**	-0.769**	1	0.697**	0.843**	0.831**	0.526*
Cr	-0.859**	0.971**	0.128	0.819**	0.173	0.890**	0.820**	-0.341	-0.930**	0.968**	-0.335	0.697**	1	0.845**	0.957**	0.493
Ni	-0.656**	0.821**	-0.324	0.455	-0.222	0.802**	0.950**	-0.728**	-0.939**	0.907**	-0.708**	0.843**	0.845**	1	0.938**	0.296
Pb	-0.801**	0.934**	-0.06	0.689**	-0.046	0.880**	0.927**	-0.531*	-0.983**	0.983**	-0.522*	0.831**	0.957**	0.938**	1	0.479
Yield	-0.508*	0.501*	0.301	0.584*	0.171	0.312	0.402	0.026	-0.389	0.452	0.018	0.526*	0.493	0.296	0.479	1

***Correlation is significant at the $P < 0.01$ level (2-tailed).*
**Correlation is significant at the $P < 0.05$ level (2-tailed).*

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Table 7

Correlations of elemental concentrations (27 May 2020) and chlorophyll fluorescence yield (Y) (30 July 2020) of energy willow (Salix triandra x Salix viminalis 'Inger') leaves in the open-field long-term experiment (Nyíregyháza, Hungary)

	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn	As	Ba	Cd	Cr	Ni	Pb	Yield
N	1	0.048	0.361	-0.148	0.156	-0.100	-0.145	0.238	-0.225	-0.099	0.166	-0.386	-0.152	-0.457	-0.196	0.511*
P	0.048	1	0.663**	0.817**	0.844**	0.850**	0.758**	0.873**	0.742**	0.929**	0.609*	0.191	0.755**	0.386	0.826**	-0.024
K	0.361	0.663**	1	0.175	0.824**	0.278	0.146	0.898**	0.144	0.476	0.822**	-0.427	0.129	-0.276	0.186	0.389
Ca	-0.148	0.817**	0.175	1	0.455	0.926**	0.960**	0.482	0.906**	0.852**	0.159	0.649**	0.950**	0.791**	0.984**	-0.376
Mg	0.156	0.844**	0.824**	0.455	1	0.524*	0.443	0.954**	0.421	0.714**	0.765**	-0.258	0.375	-0.038	0.460	0.250
Cu	-0.100	0.850**	0.278	0.926**	0.524*	1	0.874**	0.559*	0.856**	0.892**	0.306	0.509*	0.924**	0.655**	0.942**	-0.393
Fe	-0.145	0.758**	0.146	0.960**	0.443	0.874**	1	0.454	0.936**	0.848**	0.141	0.608*	0.927**	0.806**	0.960**	-0.344
Mn	0.238	0.873**	0.898**	0.482	0.954**	0.559*	0.454	1	0.446	0.742**	0.821**	-0.211	0.422	-0.017	0.498*	0.321
Zn	-0.225	0.742**	0.144	0.906**	0.421	0.856**	0.936**	0.446	1	0.884**	0.204	0.614*	0.939**	0.802**	0.937**	-0.367
As	-0.099	0.929**	0.476	0.852**	0.714**	0.892**	0.848**	0.742**	0.884**	1	0.547*	0.307	0.865**	0.528*	0.887**	-0.114
Ba	0.166	0.609*	0.822**	0.159	0.765**	0.306	0.141	0.821**	0.204	0.547*	1	-0.419	0.195	-0.274	0.195	0.411
Cd	-0.386	0.191	-0.427	0.649**	-0.258	0.509*	0.608*	-0.211	0.614*	0.307	-0.419	1	0.703**	0.870**	0.647**	-0.619*
Cr	-0.152	0.755**	0.129	0.950**	0.375	0.924**	0.927**	0.422	0.939**	0.865**	0.195	0.703**	1	0.793**	0.958**	-0.400
Ni	-0.457	0.386	-0.276	0.791**	-0.038	0.655**	0.806**	-0.017	0.802**	0.528*	-0.274	0.870**	0.793**	1	0.815**	-0.598*
Pb	-0.196	0.826**	0.186	0.984**	0.460	0.942**	0.960**	0.498*	0.937**	0.887**	0.195	0.647**	0.958**	0.815**	1	-0.366
Yield	0.511*	-0.024	0.389	-0.376	0.250	-0.393	-0.344	0.321	-0.367	-0.114	0.411	-0.619*	-0.400	-0.598*	-0.366	1

*** Correlation is significant at the $P < 0.01$ level (2-tailed).*

** Correlation is significant at the $P < 0.05$ level (2-tailed).*

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Wood ash increased the concentrations of Mn and Zn both in soils (*Table 2*) and willow leaves (*Table 4*). The correlations of these elements with Fluorescence Yield were not significant, but Zn and Y correlation was negative in all the three studied years. The correlations between Mn and Y were negative in the first year, near zero in the second year, and slightly positive in the third year (*Tables 5–7*). Zn and Mn take part in biological processes, they are essential elements for plants. Although the soil Zn and Mn concentrations were in the range of common values for uncontaminated sites, elevated Zn concentrations in willow leaves of treated cultures (*Table 4*) were around the upper limit for plants growing on unpolluted sites (SIMON, 2014). This can explain that in combination with other PTEs we calculated slightly negative correlations between Zn and Y values in each year.

Conclusions

Repeated application of wastewater solids (MSSC, MSS) and wood ash (WA) significantly enhanced the amounts of PTEs (As, Ba, Cd, Cu, Mn, Pb, and Zn) in the topsoil of willows, grown for energetical purposes in a long-term experiment. Experimental soils, however, become only mildly contaminated with PTEs, since only the concentration of As approached or exceeded the Hungarian regulatory threshold limit. It can be concluded that these substances (MSSC, MSS, or WA) can be applied repeatedly to the soil to replenish mineral nutrients, without the danger of serious soil contamination. MSSC+MSS application in combination with WA resulted in significantly higher Mn and Zn, and lower As, Ba, Cd, Cr, Ni, and Pb concentrations in topsoil than MSSC+MSS treatment without WA.

All soil treatments significantly enhanced the uptake of macronutrients (Ca, K, Mg, and P) or accumulation of PTEs in leaves of willows. Nitrogen concentrations in leaves of treated plants were generally slightly lower or similar to control. This could be explained by the ‘dilution effect’; presumably, the treated plants absorbed more nitrogen from the wastewater solids present in the soil than the control, however, this was distributed, ’diluted’ in the larger aboveground biomass.

Significantly higher leaf Mn or Zn concentrations were measured in MSSC+MSS+WA than in MSSC+MSS treatments. The assumption that WA reduces the accumulation of PTEs in willow leaves when applied altogether with MSSC and MSS was therefore only partially confirmed. It was confirmed that willows are effective phytoextractors of Cd and Zn, and moderate or low amounts of Cu, Ba, As, Cr, Mn, Pb, and Ni can be translocated from a soil amended with wastewater solids or wood ash to willow leaves.

In 2018 the treatments decreased the Fluorescence Yield values, while in 2019 and 2020 the Y values were higher in treated than in control plants. These results could be related to good macro- and micronutrient supply of treated plants’ leaves, and predicts the highest rate of photosynthesis and highest biomass accumulation of shoots in wastewater solid or wood ash treated willow cultures.

It can be also concluded that in the first year of MSS or WA application they caused slightly negative effects on the willow plants, indicated by the negative but not significant correlations between Fluorescence Yield and measured elements. It

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can be supposed that the chemical processes that have taken place in the soil caused changes in the effects of soil amendments, resulting in generally slightly positive correlations between the elements and Y in the following years. However, our results also revealed that Zn, Cd, Ni, Cu, and Fe as PTEs could have negative effects on the assimilation processes of test plants.

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