Environ Sci Pollut Res (2017) 24:4990–5000
DOI 10.1007/s11356-016-8241-5

RESEARCH ARTICLE

The evaluation of growth and phytoextraction potential of *Miscanthus x giganteus* and *Sida hermaphrodita* on soil contaminated simultaneously with Cd, Cu, Ni, Pb, and Zn

Anna Kocoń[1] · **Beata Jurga**[1]

Received: 10 June 2016 / Accepted: 11 December 2016 / Published online: 19 December 2016

Abstract One of the cheapest, environmentally friendly methods for cleaning an environment polluted by heavy metals is phytoextraction. It builds on the uptake of pollutants from the soil by the plants, which are able to grow under conditions of high concentrations of toxic metals. The aim of this work was to assess the possibility of growing and phytoextraction potential of *Miscanthus x giganteus* and *Sida hermaphrodita* cultivated on two different soils contaminated with five heavy metals simultaneously: Cd, Cu, Ni, Pb, and Zn. A 3-year microplot experiment with two perennial energy crops, *M. x giganteus* and *S. hermaphrodita*, was conducted in the experimental station of IUNG-PIB in Poland (5° 25′ N, 21° 58 ‘E), in the years of 2008–2010. *Miscanthus* was found more tolerant to concomitant soil contamination with heavy metals and produced almost double biomass than *Sida* in all three tested years, independent of soil type. *Miscanthus* collected greater amount of heavy metals (except for cadmium) in the biomass than *Sida*. Both energy crops absorb high levels of zinc, lower levels of lead, copper, and nickel, and absorbed cadmium at least, generally more metals were taken from the sandy soil, where plants also yielded better. Photosynthesis net rate of *Miscanthus* was on average 40% higher compared to *Sida*. Obtained results indicate that *M. x giganteus* and *S. hermaphrodita* can successfully be grown on moderately contaminated soil with heavy metals.

Keywords Soil contamination · Heavy metals · Energy crops · Biomass yield · Phytoextraction

Introduction

The excessive use of agrochemicals in agriculture and irrational storage of waste containing heavy metals (HMs) have led to their excessive accumulation in soil (Moosavi and Seghatoleslami 2013). Particularly high contents of HMs in the soil are found in the areas adjacent to smelters and mines (Kabala and Singh 2001; Kabata-Pendias and Mukherjee 2007). Simultaneous soil pollution with several HMs is related to geochemical properties of these elements. Dust emissions from Zn and Pb smelters bring to the soil associated geochemically with Zn and Pb Cd and smaller amounts of Cu and Ni. Exceeding the threshold values for acceptable HM content in Polish soils occurs only on several percent of the area and refers to the soils of industrial regions (Oleszek et al. 2003).

Soils contaminated with HMs require special management—reclamation, as they do not fit for typical agricultural purposes focused on food or feed production (Kabata-Pendias and Pendias 2001; Oleszek et al. 2003). Growing perennial energy crops in the areas averagely contaminated with HMs, besides the production of biomass intended for energy purposes, could also fulfill the role of land reclamation—gradually purifying the soils from these metals. Currently, studies are being carried out all over the world to examine possibilities of using different plant species for these purposes (Arshad et al. 2008; Di Baccio et al. 2003; Gisbert et al. 2006; Korzeniowska et al. 2011; Li et al. 2014; Meers et al. 2007; Mojiri 2011; Pulford and Watson 2003; Ruttens et al. 2011). Phytoextraction is one of the phytoremediation methods which uses plants for purifying the soils from both organic and inorganic impurities, especially from HMs. The

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Responsible editor: Elena Maestri

✉ Beata Jurga
bjurga@iung.pulawy.pl

Anna Kocoń
akocon@iung.pulawy.pl

[1] Department of Plant Nutrition and Fertilization, The Institute of Soil Science and Plant Cultivation - State Research Institute, Czartoryskich 8, 24-100 Puławy, Poland

mechanism behind this process involves uptaking metallic impurities by plant roots, translocating these impurities within the plant, and storing them in the aerial organs (Koopmans et al. 2007; Vassilev et al. 2004). Phytoextraction is a method which enjoys increasing popularity due to its being cost-effective, simple, and environmentally friendly (Moosavi and Seghatoleslami 2013; Susarla et al. 2002; Wrzosek et al. 2008).

The prerequisite for successful phytoextraction should be the tolerance of plants to the given soil pollutants, high "take-out" of pollutants with extraction yields due to their high concentrations or high yield, i.e., a large biomass of aerial parts (Assuncao et al. 2003; Baker and Brooks 1989; Kabala et al. 2010). A literature review on the use of perennial energy crops for reclamation of contaminated areas indicates that these plants are not typical hyperaccumulators of HMs. The degree of this purification depends on the type of pollutants, the extent of soil contamination, the species used as the phytoremediator, and plant variety (Di Baccio 2003; French et al. 2006; Laureysens et al. 2005; Liu et al. 2004; Leonardo et al. 2011; Meers et al. 2007). The advantages of using perennial crops include a long period of cultivation in one place, on average 15–20 years (Koopmans et al. 2007; Van Ginneken et al. 2007; Vangronsveld et al. 2009), and their biomass being harvested every year, as in the case of *Miscanthus* and *Sida*, or every few years in the case of willow, which promotes continuous gradual purification of soils. The technologies of phytoextraction of HMs are still at the stage of research and the selection of species, agronomic elements and methods for subsequent disposal of undesirable substances.

Salix viminalis, *Miscanthus x giganteus*, and *Sida hermaphrodita* enjoy the greatest attention among all energy crops grown in Poland due to high potential yields (Kołodziej et al. 2016; Matyka 2013). While our focus was on evaluating the annual biomass yield, *M. x giganteus* and *S. hermaphrodita* were selected for this study, because the biomass of the plants is harvested each year not as of willow every 2–3 years. The choice of the test plants was motivated also by the diversity of photosynthetic pathway of these plants (plants of type C4 and C3). Additionally, *Miscanthus* and *Sida* are representatives of two different groups of plants, monocots (*Miscanthus*) and dicotyledonous (*Sida*), so by using such a combination, we were able to incorporate different clade reaction to soil contaminated with HMs simultaneously. Most of the studies evaluating the phytoextraction abilities of plants growing on soils contaminated with one or two heavy metals. There are no wider studies on comparison of energy crops, such as *Miscanthus* and *Sida* and their yields in the areas contaminated with several HMs simultaneously.

The aim of this study was to assess the possibility of growing and phytoextraction abilities of *Miscanthus* and *Sida* cultivated on two different soils contaminated with five HMs: Cd, Cu, Ni, Pb, and Zn.

Methodology

Microplot experiment

A microplot experiment with perennial energy crops, *Miscanthus* (*M. x giganteus*) and *Sida* (*S. hermaphrodita*), was conducted in the experimental station of IUNG-PIB in Puławy (5° 25′ N, 21° 58 ‘E), Poland, in 2008–2010. The average long-term precipitation for this station is 586 mm and the mean air temperature is 7.7 °C. During the years of the research, these parameters were, respectively, 697 mm, 9.6 °C (2008), 676 mm, 8.7 °C (2009), and 551 mm and 8.2 °C (2010). The study used concrete-framed plot sized 1 × 1 × 1 m, which over 20 years ago were filled with two types of soils: loam soil (light loam) or sandy soil (weakly loamy sand). In the spring of 1994, the plots were then equally contaminated with five heavy metals: Cd, Cu, Ni, Pb, and Zn, third degree of contamination according to Kabata-Pendias et al. (1993). After the contamination of the soils, the plots were sown with plants for the first 3 years, then fallowed for 11 subsequent years until the start of this experiment.

In the spring of 2008, before the setup of the experiment, initial analyses of the soil were performed. The average contamination of the two types of soils with trace metals in the layers of 0–20 and 20–40 cm (and the average for the layer of 0–40 cm) varied, as did other properties of the soil (Table 1). In April 2008, each plot was sown with two vegetative seedlings of *Miscanthus* or *Sida* to a depth of 10–12 cm. The seedlings came from the previously grown 3–4-year-old rootstocks of these plants, from uncontaminated soils. In the first year of research, the plants were irrigated with water during the periods of drought. During the growing season, in each year of the cultivation, both plant species were fertilized with a unified dose of NPK. In the first year of cultivation, 3:2:4 g of N:P:K were used per plot, while in the two subsequent years 9:3:8 g of N:P:K per plot (Matyka 2013). The harvests of the aerial biomass of plants were carried out every year in the late autumn or winter, at a biomass moisture of about 25%. Then, the collected aerial plant biomass was dried for several days in the dryer heated to 105 °C and the yield of dry matter (DM) was determined for each plot (further in the work, referred to as biomass yield). The research results presented in this work constitute the treatment average from three replicates.

Measurements and indices of plants

During the plant growing season, in the second and third year of the experiment, the measurements of leaf gas exchange, including the intensity of photosynthesis and transpiration, and the stomatal conductance, were taken on the leaves of *Miscanthus* and *Sida* with a portable apparatus—Li-COR 6400. Water use efficiency (WUE) was calculated from the ratio of net photosynthesis intensity to transpiration intensity.

Table 1 Characteristics of experimental soil in horizontal layers 0–20 cm, 20–40 cm, and mean for 0–40-cm layer

Soil parameters	Loam			Sand		
	0–20	20–40	Mean 0–40	0–20	20–40	Mean 0–40
pH_{KCl}	6.2	6.4	6.3	6.1	6.0	6.1
SF <0.02 mm [%]	20	20	20	12	12	12
TOC [%]	0.86	0.62	0.74	1.85	0.48	1.17
P [mg kg^{-1}]	136	93	114.5	119	48	83.5
K [mg kg^{-1}]	87	54	70.5	55	45	50
Cd [mg kg^{-1}]	3.6	2.2	2.9	3.5	1.5	2.5
Cu [mg kg^{-1}]	82.5	48.8	65.7	95.9	20.4	62.3
Ni [mg kg^{-1}]	116.7	70.6	93.7	137.5	31.0	84.3
Pb [mg kg^{-1}]	769.3	415.7	592.5	709.0	145.5	427.3
Zn [mg kg^{-1}]	1215.3	797.3	1006.3	1300.0	390.0	845.0

HMs were determined as total amount

SF soil fraction, *TOC* total organic carbon

The measurements were carried out in 2009 and 2010, in spring, on the youngest, fully developed leaves of plants, in comparable stages of plant development—when the plants were 10–50 cm high, in similar environmental conditions, during the morning hours (9:00–12:00), under constant intensity of radiation of PAR 1000 μmol m^2 s^{-1}, CO_2 concentration of 390 CO_2 ppm, and air temperature 20–27 °C. Presented results consist of nine replicates from each microplot.

In addition, in the third year of cultivation (2010), in autumn at the end of the growing season before plant harvest, the following biometric measurements were taken of five plant rootstocks, randomly selected from each plot: the number of shoots per plant rootstock, the height of shoots and their diameter at 15 cm above the ground.

Chemical analyses

All the chemical analyses were performed in the Main Laboratory of IUNG-PIB in Puławy, certified by the Polish Centre of Accreditation (certificate no. AB 339) according to PN-EN ISO/IEC 17025 requirements. Before plant seeding, soils were collected from the top 20 cm and 20–40-cm layer, air-dried for 5–6 days and sieved before the analysis. The soils underwent the following determinations: soil pH; total organic carbon (TOC); the extractable fraction of P, K; total amounts of Cd, Cu, Ni, Pb, and Zn and soil texture (Table 1). The values of pH were measured potentiometrically in KCl solution using a glass electrode in a 1:5 (volume fraction) suspension of soil in water (pH in H_2O), in 1 mol l^{-1} potassium chloride solution (ISO10390: 2005). TOC was assessed by the Tiurin's method using potassium dichromate (PN-ISO14235: 2003), and texture was evaluated by the aerometric method (PN-R-04033:1998). Exchangeable cations were extracted with barium chloride solution (ISO 11260), and their content was determined by flame atomic absorption spectrometry (FAAS). The Cd, Pb, Cu, Ni, and Zn in extracts were determined by FAAS after mineralization in aqua regia according to PN-ISO 11047:2001P. The concentrations of HMs in aerial plant material previously ashed in a muffle furnace at 500 °C and dissolved in 20% nitric acid (PN-R-04014: 1991) were determined by flame atomic absorption spectrometry (FAAS).

Bioaccumulation factor (BF) of Cd, Cu, Pb and Zn (in 2008, the concentration of Ni in plants was not determined) in plant tissues was calculated for 2008, from the first harvest of plant biomass, from the ratio of the concentration of these metals in plant biomass to the concentration of these metals in the soil, according to following equation:

$$BF = \frac{\text{metal concentration in aboveground organs } \left(\text{mg kg}^{-1}\right)}{\text{metal concentration in soil } \left(\text{mg kg}^{-1}\right)}$$

Statistical analyses

Data are presented as means and standard errors. The analysis of variance (ANOVA) was realized. The Tukey HSD test was performed for pair-wise comparison ($p < 0.05$). All statistic tests were performed using Statistica 12 software.

Results

Biomass yield

The yield of the biomass of *M. x giganteus* and *Sida hermaphrodita* depended on plant species, the age of the plantation, soil type, and properties, and the degree of its contamination with HMs (Table 2). The yields of both plants were the lowest in the first year of the experiment. In the second year,

Table 2 Plant yields—biomass of aerial parts of plants in the years (g DM·m^{-2})

Plant	Soil	2008	2009	2010
Miscanthus x giganteus	Loam	184.6 ± 18.1b	675.9 ± 211.4b	1699.3 ± 195.3c
	Sand	478.0 ± 68.1a	1935.5 ± 151.5a	4280.0 ± 95.0a
Sida hermaphrodita	Loam	74.0 ± 0.1c	278.3 ± 150.6c	621.3 ± 193.1d
	Sand	483.9 ± 15.0a	1551.8 ± 20.3a	2251.1 ± 123.4b

Values are expressed as mean ± S.E. Values in columns followed by the same letters did not differ significantly according to Tukey's test ($P < 0.05$)

DM dry matter

they increased, being the greatest in the third year of the experiment. Both *Miscanthus* and *Sida* always yielded better on sandy soil compared to loamy soil. For the 3 years of research, biomass yield in sandy soil was respectively 2.6- and 4.4-fold greater than compared to loamy soil. The analysis of biometric features revealed that for both *Miscanthus* and *Sida*, tested parameters were always higher from microplots filled with sandy soil, but not all differences were statistically significant (Table 3). *Miscanthus* turned out to be more tolerant of total contamination than *Sida* and yielded better, in all the 3 years, regardless of the type of soil, providing nearly twice the biomass yield (Table 2).

Photosynthetic activity of plants

Photosynthetic activity of plants was evaluated by the parameters of gas exchange: net photosynthesis rate, transpiration rate, stomatal conductance, and water use efficiency in 2 years (2009–2010). The values varied among plant species. Net photosynthesis rate of *Miscanthus* leaves varied in the 2 years of study, while for *Sida*, the intensity of this process was similar (Table 4). Net photosynthesis rate was always higher for *Miscanthus* than for *Sida*, on average by 40% for 2 years and for sandy soil compared to loam (on average by 20%).

Leaf transpiration rate, stomatal conductance, and water use efficiency were in fairly wide range values within the plants, soils, and years of research (Table 4). Transpiration rate of *Miscanthus* leaves was lower than of *Sida*. Irrespective of the tested plant, a transpiration rate was higher and statistically significant for plants growing on the sand in relation to ones grown on loam. The rate of WUE was more favorable for *Miscanthus* than for *Sida*, reaching higher values for plants grown on loamy soil compared to the sandy ones, which was statistically proven. As for stomatal conductance, higher values were observed for *Sida* than for *Miscanthus*, higher values of this parameter were noticed for plants grown on sandy rather than loamy soil (Table 4).

Concentrations of Cd, Cu, Ni, Pb, and Zn in plant biomass

The concentrations of Cd, Cu, Ni, Pb, and Zn in the biomass were within wide ranges and depended on the element, plant, soil type, and year of cultivation (Table 5). Concentrations of the metals in the biomass of plant tissues were highest for Zn, followed by Pb, Cu, Ni, and Cd. The largest concentration of these metals (except for Ni, which was not determined in the first year) in both plant species occurred in the first year of their cultivation. Generally, *Sida* contained more metals than *Miscanthus*, grown on loamy soils compared with sandy soils. In the second year (2009), the concentrations of metals in plant tissues were lower and usually were the lowest in the oldest plants from the 2010 harvest.

Analyzing the bioaccumulation factor of Cd, Cu, Zn, and Pb (Table 6) in an aerial plant biomass in the first year of the research showed that on loamy soils, it was always higher for *Sida* than for *Miscanthus*. In the case of sandy soil, the accumulation of metals in the plants varied and did not follow the pattern observed for loamy soil. In addition, regardless of plant species, indicators were always larger for Zn than for Cd, compared with the other two metals—Cu and Pb.

Table 3 Chosen biometric features of plants in year 2010

Plant	Soil	Number of shoots per plant	Shoot diameter (mm)	Shoot length (cm)
Miscanthus x giganteus	Loam	43.0 ± 7.0b	8.6 ± 0.3b	177.2 ± 10.7b
	Sand	69.0 ± 5.5a	10.4 ± 0.7b	272.5 ± 8.1b
Sida hermaphrodita	Loam	6.0 ± 2.0c	15.0 ± 5.9b	222.6 ± 77.3b
	Sand	15.0 ± 1.5c	24.1 ± 1.4a	366.0 ± 6.1a

Values are expressed as mean ± S.E. Values in columns followed by the same letters did not differ significantly according to Tukey's test ($P < 0.05$)

Table 4 Net photosynthesis rate (P_N), transpiration rate (E), water use efficiency WUE (ratio between net photosynthesis rate and transpiration rate), and stomatal conductance (G_s) leaves of plants years 2009 and 2010

Plant	Soil	2009	2010
P_N (μmol CO_2.m^2 s^{-1})			
Miscanthus x giganteus	Loam	21.5 ± 2.2a	11.9 ± 1.7c
	Sand	23.1 ± 0.4a	17.3 ± 0.2a
Sida hermaphrodita	Loam	12.2 ± 1.8c	10.8 ± 0.8c
	Sand	13.9 ± 2.0b	14.9 ± 0.6b
E (mmol H_2O m^2 s^{-1})			
Miscanthus x giganteus	Loam	2.4 ± 0.4c	1.4 ± 0.2c
	Sand	3.8 ± 0.1b	2.0 ± 0.0b
Sida hermaphrodita	Loam	3.1 ± 0.7c	2.0 ± 0.3b
	Sand	6.5 ± 0.2a	4.9 ± 0.2a
WUE (P_N/E)			
Miscanthus x giganteus	Loam	8.9 ± 0.5a	8.5 ± 0.6a
	Sand	6.1 ± 0.1b	8.7 ± 0.1a
Sida hermaphrodita	Loam	4.0 ± 0.4c	5.4 ± 0.8b
	Sand	2.1 ± 0.3d	3.0 ± 0.2c
G_s (mmol H_2O m^2 s^{-1})			
Miscanthus x giganteus	Loam	0.153 ± 0.0b	0.110 ± 0.0b
	Sand	0.144 ± 0.0b	0.136 ± 0.0b
Sida hermaphrodita	Loam	0.180 ± 0.1b	0.117 ± 0.0b
	Sand	0.474 ± 0.1a	0.244 ± 0.0a

Values are expressed as mean ± S.E. Values in columns followed by the same letters did not differ significantly according to Tukey's test ($P < 0.05$)

Accumulation of Cd, Cu, Ni, Pb, and Zn in plant biomass

Under the conditions of this study, both plants accumulated Zn in the highest concentrations compared to Pb, Ni, and Cu, while Cd was the lowest of all (Table 7). Larger quantities of Zn were accumulated by plants growing on sandy soil. *Miscanthus* accumulated the most of the Zn in the second year of cultivation—355.8 mg Zn per plot (mg Zn m^{-2}) compared to *Sida* in the first year of its cultivation—79.9 mg. In the 3-year period, *Miscanthus* accumulated three times more Zn than *Sida*, on average by 468.3 and 160.6 mg Zn (Fig. 1). As compared with Zn, the amounts of accumulated Pb were much lower, averagely by 60-fold for *Miscanthus* and 26-fold for *Sida* (Fig. 2). The highest amounts of Pb were accumulated in plant biomass in the first year of the cultivation (Table 7), from 3.0 to 5.7 mg Pb per plot, larger on loamy soil than on sandy soil. *Miscanthus* took up on average 9.0 mg Pb, whereas *Sida* uptake was smaller and amounted to 6,2 mg on average per plot in three years of the cultivation (Fig. 2).

Tested plants extracted small amounts of Ni, greater amounts were taken by *Miscanthus* (9.2 mg) than *Sida* (1.2 mg Ni per plot), the most in the third year of cultivation. Similar results were found for Cu as both plants took up approximately 50% of total uptake in the third year of cultivation. Total extraction of Cu was higher for plants cultivated on sandy soil. Cd was uptaken in the lowest concentration, *Miscanthus*'s uptake was 0.65 mg Cd per plot, and *Sida*'s—0.95 mg Cd per plot.

Discussion

Phytoextraction is a phytoremediation technique that relies on the use of plants to extract and translocate metals. The shoots are subsequently harvested to remove the contaminants from the soil to achieve regulatory levels within a reasonable time frame (Moosavi and Seghatoleslami 2013; Nascimento and Xing 2006; Peng et al. 2009). Growth and development of plants on soils contaminated with HMs depends on many factors, but primarily on the concentration of these metals in the soil, tolerance of the phytoremediating species, the interaction between metals, and the physiochemical properties of the soil, such as pH, organic matter content, or phosphorus concentration (Arshad et al. 2008; Assuncao et al. 2003; Di Baccio et al. 2003; Kabala and Singh 2001; Kabata-Pendias and Pendias 2001; Liu et al. 2004; Meers et al. 2007; Spiak 1998; Sękara et al. 2005; Waterlot et al. 2011; Yoon et al. 2006).

Tolerance to heavy metals

The tolerance of particular energy crops to selected environmental contaminants is currently being widely tested (Korzeniowska and Stanislawska-Glubiak 2015; Meers et al. 2010; Pandey et al. 2016; Techer et al. 2012). It varies depending on a plant species, or even a cultivar grown under different climatic conditions (Korzeniowska et al. 2011; Wrzosek et al. 2008). As Malik et al. (2010) found, grasses are generally more effective in phytoremediation than bushes or trees, due to a substantial growth rate, greater abilities to adapt to the environment and stresses, and high biomass yield. This is reflected in the results of this study, as *M. x giganteus* turned out to be more tolerant compared to *S. hermaphrodita* evidenced by higher net photosynthesis rate, larger yields, and greater HM accumulation. The two tested genotypes of plants grown in Poland on uncontaminated soils vary in terms of biomass yield as well.

In this study, *Miscanthus* yielded better than *Sida*. Moreover, both plants yielded less in the first year in relation to subsequent years of their cultivation, which corresponds to literature findings (Matyka 2013). This is attributed to the fact that the youngest plants grown on soil contaminated with HMs are more sensitive to stress associated with an excess of these metals than older plants (Baker 1981; Mleczek et al. 2009). In this study, high concentrations of these metals in the 0–20-cm soil layer, especially during the first year of cultivation, significantly reduced the growth and development of

Table 5 The concentrations of Cd, Cu, Ni, Pb, and Zn in the plant biomass (in mg kg^{-1}DM)

Plant	Soil	2008	2009	2010
		mg	mg	mg
		Cd		
Miscanthus x giganteus	Loam	0.8 ± 0.1b	0.2 ± 0.1b	0.1 ± 0.0c
	Sand	0.5 ± 0.0c	0.2 ± 0.0b	0.1 ± 0.0c
Sida hermaphrodita	Loam	2.4 ± 0.1a	0.5 ± 0.0a	0.4 ± 0.0a
	Sand	0.7 ± 0.0b	0.2 ± 0.0b	0.2 ± 0.0b
		Cu		
Miscanthus x giganteus	Loam	8.9 ± 0.2a	1.3 ± 0.3b	1.3 ± 0.2c
	Sand	3.5 ± 0.1b	0.8 ± 0.0c	0.9 ± 0.1d
Sida hermaphrodita	Loam	12.2 ± 3.5a	2.0 ± 0.2a	1.9 ± 0.0a
	Sand	2.6 ± 0.0b	1.5 ± 0.1b	1.6 ± 0.1b
		Ni		
Miscanthus x giganteus	Loam	–[a] –	3.2 ± 0.8a	4.7 ± 0.9a
	Sand	– –	1.4 ± 0.2b	1.7 ± 0.4b
Sida hermaphrodita	Loam	– –	0.7 ± 0.1c	0.4 ± 0.0c
	Sand	– –	0.3 ± 0.1c	0.3 ± 0.1c
		Pb		
Miscanthus x giganteus	Loam	38.5 ± 0.7b	0.9 ± 0.3b	1.2 ± 0.4a
	Sand	7.8 ± 0.4c	1.6 ± 0.1a	0.7 ± 0.1ab
Sida hermaphrodita	Loam	83.6 ± 30.5a	1.2 ± 0.1ab	0.5 ± 0.0b
	Sand	6.1 ± 0.0c	0.8 ± 0.0c	0.6 ± 0.1b
		Zn		
Miscanthus x giganteus	Loam	305.0 ± 57.0b	177.7 ± 28.4a	83.6 ± 8.1a
	Sand	228.0 ± 9.0 cd	184.0 ± 2.0a	88.2 ± 13.8a
Sida hermaphrodita	Loam	937.5 ± 80.5a	41.6 ± 8.7c	26.0 ± 3.2b
	Sand	165.5 ± 9.5d	48.2 ± 10.7bc	29.0 ± 2.4b

Values are expressed as mean ± S.E. Values in columns for the same microelement followed by the same letters did not differ significantly according to Tukey's test ($P < 0.05$)

[a] In 2008, the concentration of Ni in plants was not determined

plants, and ultimately, the biomass yield. In subsequent years, higher biomass yields were obtained, as the conditions for plant growth improved, due to the pollutants being removed from the soil. It seems that a deep root system of older plants outgrew the upper, most contaminated soil layer and reached deeper into the less contaminated soil. Similar relationships regarding the biomass production of *Miscanthus* and *Sida* grown on soils polluted with Zn and Pb were obtained by Kocon and Matyka (2012) and for willow cultivated on soils contaminated with Zn, Cd, and Pb by Stanislawska-Glubiak et al. (2012).

Table 6 Bioaccumulation factor (BF) of Cd, Cu, Pb, and Zn in plant biomass for 2008

Plant	Soil	Cd	Cu	Pb	Zn
Miscanthus x giganteus	Loam	0.28	0.14	0.06	0.30
	Sand	0.20	0.06	0.02	0.27
Sida Hermaphrodita	Loam	0.83	0.19	0.14	0.93
	Sand	0.28	0.04	0.01	0.20

Photosynthesis rate measured in 2 years was higher for *Miscanthus* compared to *Sida* (Table 4). *Miscanthus* plants not only showed a higher net photosynthesis rate but also a lower transpiration rate in relation to *Sida*, which led to a better water use. Its WUE was significantly higher and more favorable, which resulted in higher yields. According to Matyka (2013), *M. x giganteus* is characterized by efficient water use, and its transpiration rate is low, amounting to 250–340 l kg^{-1} of DM. This author also claims that in Central and Eastern Europe conditions, due to large yields, the annual total rainfall should not be lower than 500–600 mm, consistent with the conditions during this experiment. The high photosynthetic efficiency of *M. x giganteus*, compared to other C4, and C3 plants, under adverse environmental conditions, including low temperatures was highlighted by Farage et al. (2006) and Naidu and Long (2004).

Table 7 The accumulation of Cd, Cu, Ni, Pb, and Zn in the plant biomass (mg DM m^{-2}) depending on soil and study year

Plant	Soil	2008		2009		2010	
		mg	%	mg	%	mg	%
			Cd				
Miscanthus x giganteus	Loam	0.1 ± 0.0b	29.5	0.2 ± 0.1b	35.6	0.1 ± 0.0b	34.9
	Sand	0.2 ± 0.0ab	24.5	0.3 ± 0.0a	35.3	0.4 ± 0.0a	40.2
Sida hermaphrodita	Loam	0.2 ± 0.0ab	18.3	0.3 ± 0.1a	35.5	0.4 ± 0.1a	46.2
	Sand	0.4 ± 0.1a	32.0	0.4 ± 0.0a	33.5	0.4 ± 0.0a	34.5
			Cu				
Miscanthus x giganteus	Loam	1.3 ± 0.1a	29.2	0.9 ± 0.4b	20.2	2.3 ± 0.1b	50.6
	Sand	1.7 ± 0.2a	25.5	1.5 ± 0.1b	23.2	4.3 ± 0.1a	57.3
Sida hermaphrodita	Loam	1.1 ± 0.3b	24.5	1.3 ± 0.5b	30.5	2.0 ± 0.4b	45.0
	Sand	1.3 ± 0.0a	17.7	2.4 ± 0.1a	33.0	3.6 ± 0.5a	49.3
			Ni				
Miscanthus x giganteus	Loam	–	–	2.1 ± 1.0a	23.1	7.1 ± 0.1a	76.9
	Sand	–	–	2.7 ± 0.6a	31.2	6.0 ± 1.1a	68.8
Sida hermaphrodita	Loam	–	–	0.4 ± 0.1b	47.1	0.5 ± 0.1b	52.9
	Sand	–	–	0.5 ± 0.1b	40.3	0.7 ± 0.3b	59.7
			Pb				
Miscanthus x giganteus	Loam	5.7 ± 0.6a	67.5	0.4 ± 0.2c	5.1	2.3 ± 0.7a	27.4
	Sand	3.7 ± 0.4b	39.6	3.0 ± 0.0a	32.3	2.6 ± 0.3a	28.1
Sida hermaphrodita	Loam	5.6 ± 0.3a	81.6	0.8 ± 0.3b	11.4	0.5 ± 0.1c	7.0
	Sand	3.0 ± 0.1b	53.3	1.2 ± 0.0b	21.2	1.4 ± 0.2b	25.5
			Zn				
Miscanthus x giganteus	Loam	4.5 ± 3.2d	15.1	113.5 ± 19.7b	38.4	137.2 ± 3.3b	46.5
	Sand	10.6 ± 19.8c	14.0	355.8 ± 24.0a	45.6	315.0 ± 37.3a	40.4
Sida hermaphrodita	Loam	44.6 ± 9.4b	44.2	28.4 ± 14.3d	28.2	28.0 ± 9.4c	27.7
	Sand	79.9 ± 2.4a	36.3	74.6 ± 15.6c	33.9	65.6 ± 9.0c	29.8

Values are expressed as mean ± S.E. Values in columns for the same microelement followed by the same letters did not differ significantly according to Tukey's test ($P < 0.05$)

According to simulations carried out for Eastern Europe, the yields of *Miscanthus* dry mass, on very good soils can range from 17.7 to 21.8 t ha^{-1} (Fischer et al. 2005). Kołodziej et al. (2016) in a field experiment with sewage sludge application obtained even higher *Miscanthus's* yields amounting to 25 t ha^{-1}. In long-term research on *Miscanthus* grown in uncontaminated loamy soil in the same period (2008–2011) and in analogous geographical position, Matyka (2013) obtained similar results of *Miscanthus* and

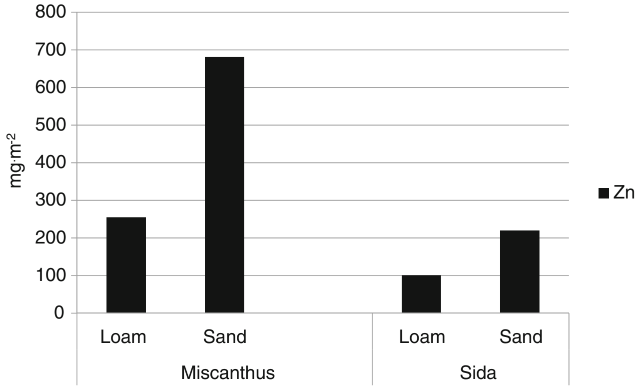

Fig. 1 The sum of uptake of Zn (mg m^{-2}) by *Miscanthus x giganteus* and *Sida hermaphrodita*

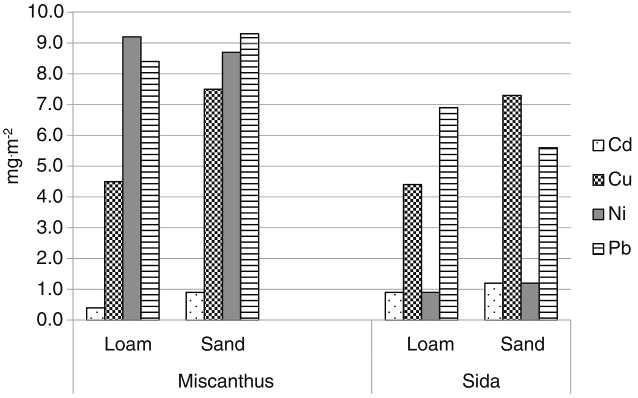

Fig. 2 The sum of uptake of Cd, Cu, Ni, and Pb (mg m^{-2}) by *Miscanthus x giganteus* and *Sida hermaphrodita* cultivated on loamy and sandy soil for 2008–2010

Sida yield compared to this study. Biometric characteristic corresponded to these of this study as well. Matyka (2013) proved that *Miscanthus* cultivation was cost-effective for uncontaminated soils. Therefore, taking into account the benefits of purified soil, aesthetical values and provided the obtained raw material would meet the basic requirements to be used for energy purposes, the economic balance of phytoextraction of moderately polluted soil in our simulated conditions could be positive also.

Heavy metal uptake

The highest concentrations of HMs in plant tissues were determined in the first year of the study and declined gradually each year, which could be attributed to dilution in a larger biomass. Results of this study for Pb accumulation in aerial parts corresponds well with findings of Wanat et al. (2013) who observed that shoot and leaves of *Miscanthus* grown on soil polluted with Sb, As, and Pb accumulate in total from 0.6 to 43 mg Pb.kg^{-1} while in our experiment, the Pb accumulation varied from 0.7 to 38.5 mg Pb.kg^{-1}. Nsanganwimana et al. (2015) tested different *Miscanthus* cultivars grown on soil contaminated with Cd, Pb, and Zn with addition of inoculum of arbuscular mycorrhizal fungi. After second growing season, shoots of *Miscanthus* accumulate greater amounts of Zn, smaller quantities of Pb and Cd (from 80 to 127 mg kg^{-1} for Zn, from 1.5 to 2.0 mg kg^{-1} for Pb and from 1.0 to 1.7 mg kg^{-1} for Cd). Cd and Pb concentrations in shoot yield observed by Nsanganwimana et al. (2015) are higher than observed in this study after second growing season, only Zn concentration is lower. This could be attributed to higher initial soil HM concentration and addition of mycorrhizal inoculum.

In this study, despite the relatively large concentrations of Pb in the soil (Table 1), plants collected minor quantities of this element. Taking into account the results of Liu et al. (2004), Moosavi and Seghatoleslami (2013), Nsanganwimana et al. (2015), Sękara et al. (2005), Stanislawska-Glubiak et al. (2015), and Yoon et al. (2006), it could be explained by deposition of Pb in plant's roots.

The measure of mobility and availability of HMs for a plant is the bioaccumulation factor (BF). By analyzing the BF of Cd, Cu, Pb, and Zn (Table 6) in the biomass from the first year, it can be concluded that values were higher for *Sida* than for *Miscanthus*. Kabata-Pendias and Pendias (2001) and Kuboi et al. (1986) report that dicotyledons which include *Sida* accumulate higher amounts of toxic HMs in the aerial parts of plants with respect to the monocots, which include *Miscanthus*. Regardless of tested plant species, BF was always higher for Zn and Cd than Cu and Pb. Higher rates for Zn and Cd are probably a consequence of the greater ability of these elements to accumulate in the aboveground mass of plants than in roots, as reported by Korzeniowska et al. (2011) and Yoon et al. (2006). The BF value in the shoots of accumulator plants according to McGrath and Zhao (2003) should be higher than in "normal" plants and exceed 1. Results of study suggest neither *Miscanthus* nor *Sida* can be classified as an accumulator plant as the highest BF found in *Sida* on loamy soil was 0.83 for Cd and 0.93 for Zn.

Impact of soil type

Results with *Miscanthus* and *Sida* cultivation on two contaminated soils, deviating in terms of physical and chemical properties, showed considerable differences. On average, loamy soil was characterized by higher concentrations of all elements (apart from organic matter content) compared to the sandy soil (Table 1). According to Kabata-Pendias and Pendias (2001), Pb and Zn occurred in both soils in toxic amounts, while Cd, Cu, and Ni in increased concentrations. Results showed that both *Sida* and *Miscanthus* yielded higher growth rates on sandy soil than on loam, corresponding to results obtained by Faber et al. (2008), who attributed increased biomass production to faster soil heating. These findings are supported by Matyka (2013), who discovered that, under favorable thermal conditions and appropriate rainfall, *Miscanthus* plants yielded higher on lighter soils than on heavy soils. The analysis of the selected biometric features of plants carried out in the third year of research showed that a yield increase on sandy soil was elicited by a higher number of shoots and a larger diameter and length of shoots (Table 3).

Studies on the intensity of net photosynthesis and biomass production showed more severe disturbances in the intensity of these processes in both plant species when grown on loamy soil compared to sandy soil. This was related to a higher reduction of the main process responsible for yields, i.e., the intensity of net photosynthesis, and in turn, could be associated with higher concentrations of HMs in loamy soil. It is known that an excess of HMs adversely affects the physiological processes of plants, including photosynthesis (Schmidt 2003; Moosavi and Seghatoleslami 2013). According to Burzynski and Klobus (2004) and Zhang et al. (2012), HMs reduce the content of chlorophyll in plants and interfere and limit the activity of photosystems I and II. Disturbances in the activity of photosynthesis in maize due to soil contamination with Cd were recorded by Zhang et al. (2012), while in willow and maize under Cd, Pb, and Zn stress by Stanislawska-Glubiak et al. (2012, 2015).

The metal content in the soil and organic matter content influence the concentration of HMs in the plant. However, the role of organic matter is not clear, as results show that application of organic matter as a support in phytoremediation stimulates coniferous trees to collect greater amounts of Cd but smaller amounts of Pb (Placek et al. 2016). Minor accumulation could be attributed to that with a significant presence of organic matter, heavy metals are to a greater extent adsorbed by the soil complex, immobilized and less accessible for a plant (Pikuła and Stepien 2007; Chłopecka and Adriano 1997; Nawab et al. 2016). The results of Nawab et al. (2016) suggest that the type of organic matter has a significant influence on the behavior of HMs in the soil and efficiency of phytoremediation. He concludes that this issue requires further research to draw definite conclusions.

The potential of using energy crops from phytoremediation for the energy production

As a consequence of results of research on yield of *Miscanthus* cultivated on contaminated sites (Lewandowski et al. 2000; Heaton et al. 2010; Wanat et al. 2013; Wisz and Matwiejew 2005; Brosse et al. 2016; Pandey et al. 2016), Europe increasingly sees the potential of using *Miscanthus* as a biofuel. This is related to the fact that currently, the use of renewable energy sources at EU level is regulated by a European Parliament and Council Directive 2009/28/EC (2009) on the promotion of energy from renewable sources. This document obliges the EU Member States to increase the share of renewable energy in total energy consumption of 20% by 2020. With respect to adopted regulation, the demand for renewable energy resources is growing, both in Poland and in Europe. However, there is serious competition with agricultural production. This conflict can be avoided if the cultivation of energy crops on land unsuitable for agricultural production can be economically justified. Not only EU countries recognize the potential of using energy crops for remediation purposes. The United States Environmental Protection Agency has identified 66,000 locations where contaminated lands should be subjected for phytoremediation by energy crops (Pandey et al. 2016).

Concentrations of HMs in the biomass in the first year of this study exceeded their natural content (Kabata-Pendias and Pendias 2001) as well as the acceptable heavy metal threshold for energy crops set out for granulated fuels from natural wood in Germany DIN 51731. This standard allows for a Cd content of <0.5 mg kg^{-1}, Cu <5 mg kg^{-1}, Pb <10 mg kg^{-1}, and Zn <100 mg kg^{-1} (it has no threshold for Ni). In the second year of phytoextraction, only Zn content in *Miscanthus* biomass exceeded the threshold value while in the third year, both *Miscanthus* and of *Sida* yields met standards. It can be assumed that since the third year of phytoremediation harvested biomass could be a clean, carbon-neutral and eco-friendly source of renewable energy. If the biomass produced from the phytoextraction of contaminated sites does not fit safety threshold, it has other opportunities for use, i.e., in pulp and fiber production (Marin et al. 2009; Pandey et al. 2016), this could be used for production of fiber-boards, building blocks, and composite particle boards (Lewandowski et al. 2000, Nsanganwimana et al. 2014). A literature study reveals that a side effect of *Miscanthus* cultivation is an increase in the soil organic matter, stimulation of soil microbial activity, and diversity (Anderson-Teixeira et al. 2009, Techer et al. 2012). *M. x giganteus* does not produce viable seed and so there is no chance of becoming invasive species.

One of the challenges in the study of phytoremediation in controlled, laboratory conditions is that stress factors such as variations in temperature, precipitation, nutrients and herbivory (insects and/or animals), and plant pathogens cannot be taken into account. The results of studies from soils artificially contaminated with a single metal do not reflect conditions in multicontaminated soils. Nascimento and Xing (2006) found that 70% of all metal-contaminated sites in the USA involve two or more metals. Consequently, results from studies with a singular pollutant may not be sufficient to make conclusion for remediation measures on that sites. Thus, the advantage of this microplot study and the results is the consideration of both concomitant soil pollution and abiotic as well as biotic stresses. This enhances the credibility of the results and their reproducibility in practice.

Conclusions

Perennial energy crops, which include *M. x giganteus* and *S. hermaphrodita* can be grown on moderately contaminated soils with several HMs. *Miscanthus* is more tolerant to the total contamination of Cd, Cu, Ni, Pb, and Zn than *Sida* which was evidenced by the higher yields and greater accumulation of HMs, except for Cd.

A comparison of the influence of soil type revealed that both plant species yielded higher biomass levels and accumulated higher amounts of HMs when grown on sandy soil than on clay soils. Greater phytoextraction potential to Zn, Pb, Cu, Ni, with the exception of Cd was found for *Miscanthus* compared to *Sida*. Plants effectively purified soil from Zn but uptook lower levels of Pb, Cu, Ni, and least of Cd.

Based on the results, it is concluded that *Miscanthus* could meet two essential demands: phytoremediation of contaminated sites and increasing biofuel needs, being a part of a solution for achieving economic and environmental sustainability.

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