DOI: 10.1007/s11099-018-0827-3 PHOTOSYNTHETICA 56 (4): 1249-1258, 2018

Zinc accumulation, photosynthetic gas exchange, and chlorophyll *a* fluorescence in Zn-stressed *Miscanthus* × *giganteus* plants

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Abstract

Accumulation and distribution of zinc within *Miscanthus* × *giganteus* plants grown on elevated Zn concentrations and their photosynthetic performance were investigated. High concentrations of Zn in soils caused an increase of its concentrations in all plant organs. The bioconcentration factor, bioaccumulation factor, and translocation factor were lower than one indicating that *M.* × *giganteus* is an excluder plant species. Excessive Zn induced visible leaf damage, *i.e.* chlorosis and necrosis, only in the oldest leaves, pointing to Zn accumulation. Elevated amounts of Zn in leaves significantly lowered the photosynthetic rate, transpiration rate, stomatal conductance, intercellular CO_2 concentrations, parameters of chlorophyll *a* fluorescence, and chlorophyll *b* content. Despite Zn excess in leaves, there was no severe reduction in the maximal quantum yield of PSII photochemistry, indicating a high photosynthetic capacity, high tolerance to elevated Zn concetrations, and ability of *M.* × *giganteus* to grow on Zn-contaminated soils.

Additional keywords: pigments; photosynthesis; phytoremediation; Zn tolerance.

Introduction

Zinc is a ubiquitous chemical element, the average content of which in the Earth's crust is 70 mg kg^{-1} (Kabata-Pendias 2011), whereas its concentration in world soils can vary between 10 and 100 mg kg^{-1} (Martens and Smolders 2013). The availability of Zn for plant uptake depends on its concentration in the soil, the soil clay fraction, and pH of the soil. Zinc is slightly mobile and poorly available at alkaline pH and in soils with a high clay fraction due to strong Zn binding (Kabata-Pendias 2011, Rutkowska *et al.* 2013, Gajić *et al.* 2016). Excessive concentrations of Zn in soils naturally result from the chemical and physical weathering of parent rocks rich in Zn. High concentrations of Zn in soil frequently derive from anthropogenic input through mining and smelting operations, treatment of agricultural soils with Zn fertilizers, and combustion of fossil fuels in urban environments (Pilgrim and Hughes 1994, Kabata-Pendias 2011, Martens and Smolders 2013).

Zinc is an essential element for plants and acts as a catalytic or structural cofactor of a large number of enzymes and regulatory proteins, and it is involved in metabolism of carbohydrates, proteins, and phosphates (Marschner 1995, Kabata-Pendias 2011). High concentrations of Zn can damage cell functioning due to the displacement of other elements having similar diameter and charge, and they can also disrupt a number of essential processes in the plant organism. Thus, Zn excess reduces plant growth and induces chlorosis of leaves, which can be a result of Zn-induced Fe or Mg deficiency (Marschner 1995, Sagardoy *et al.* 2009). Moreover, it decreases leaf water content, transpiration rates, stomatal conductance, the net photosynthetic rate, and photosynthetic efficiency (Bonnet *et al.* 2000, Sagardoy *et al.* 2009). Although the toxicity thresholds for Zn widely depend on the plant species, they can also be highly variable between

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Received 31 August 2017, *accepted* 10 January 2018, *published as online-first* 21 June 2018.
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Abbreviations: BAF – bioaccumulation factor; BCF – bioconcentration factor; Car – carotenoids; Chl – chlorophyll; C_i – intercellular CO_2 concentration; DM – dry mass; E – transpiration rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_v – variable fluorescence; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; P_N – photosynthetic rate; R – respiration; TF – translocation factor.
Acknowledgements: The authors gratefully acknowledge financial support from the Serbian Ministry of Education, Science, and Technological Development (grant No. 173030 and grant No. 173018). We thank the reviewers and the editors for their valuable suggestions and comments. We would also like to thank Mr. Raymond Dooley for editing the English language of the manuscript.

different growth stages of the same species. Toxicity symptoms in sensitive plants become usually visible at 150−200 mg(Zn) kg^{-1}(dry mass, DM) (Kloke *et al.* 1983). Most commonly, the toxic Zn concentrations in various plants range from 100 to 500 mg(Zn) kg^{-1}(DM), and toxic effects are manifested as stunted plant growth and leaf chlorosis (Marschner 1995).

Miscanthus × *giganteus* (Greef & Deuter) (Poaceae) is a perennial and rhizomatous C$_4$ plant that produces high biomass under temperate climatic conditions (Naidu and Long 2004). It is undemanding in regard to nutrient supply, is able to grow on a wide range of soils, and grows with minimal costs in one location for a long period of time (15–20 years), due to which its cultivation is highly economical (Zub and Brancourt-Hulmel 2010, Dželetović *et al.* 2013, McCalmont *et al.* 2017). It is cultivated as a renewable energy source, but its production is restricted to marginal lands. Studies focused on the phytoremediation potential of *M.* × *giganteus* show its potential to grow on soils contaminated by heavy metals or organic pollutants (Técher *et al.* 2012, Wanat *et al.* 2013, Nsanganwimana *et al.* 2014, Pavel *et al.* 2014, Pidlisnyuk *et al.* 2014, Bang *et al.* 2015, Figala *et al.* 2015, Pelfrêne *et al.* 2015, Pandey *et al.* 2016).

There is a significant body of published data related to the physiological and biochemical response of *Miscanthus* to low temperatures (Beale *et al.* 1996, Głowacka *et al.* 2015, Fonteyne *et al.* 2016, Jiao *et al.* 2016, 2017), the effect of different light intensity (Sun *et al.* 2012, 2014; Ubierna *et al.* 2013, Ma *et al.* 2017), water deficit (Clifton-Brown *et al.* 2002, Ings *et al.* 2013), influence of the bacterial community (Técher *et al.* 2012), the effect of arbuscular mycorrhizal fungal inoculation (Firmin *et al.* 2015), and usefulness of fertilizers (Ma *et al.* 2017, Pogrzeba *et al.* 2017). However, aspects of the photosynthetic response of *Miscanthus* to contamination by different metals still remain far from being completely understood.

High leaf concentrations of Zn may adversely affect the amount of pigments, gas exchange, and photosynthetic activity in this plant species. Accordingly, the aims of this study were as follows: (*1*) determination of Zn concentrations in the soil and in plant organs in order to assess the phytoremediation potential of *M.* × *giganteus*; and (*2*) assessment of the effects of different leaf concentrations of Zn on the pigment content and various photosynthetic parameters.

Materials and methods

Plant material and growing conditions: The rhizomes of *M.* × *giganteus* used in this experiment were collected from the field at the Institute for Application of Nuclear Energy (INEP), Zemun, Serbia (44°51´N, 20°22´E), where plants have grown for years. Rhizomes with several auxiliary buds were cut from dormant mother plants and planted in pots under greenhouse conditions in March 2016. The pot experiment was conducted in facilities of the INEP in Zemun, Serbia. Two plant rhizomes (7–10 cm long) with several buds were planted per each 10-L pot in soil with a depth of 10 cm. Plants were grown for one month (March) in unpolluted soil under greenhouse conditions (natural light conditions, minimum–mean–maximum daily temperature of 10.1–13.3–16°C, respectively). After the first month of growth, plants were subjected to single treatment with different concentrations of $ZnSO_4 \times 7\ H_2O$:

Treatment	[mg(Zn) kg^{-1}(DM)]
Control	0
T_{250}	250
T_{500}	500
$T_{1,000}$	1,000

Plants in pots were then transferred to the field. In the course of the next three months, all plants were exposed to natural light conditions (direct sunlight: April – 200.8 h, May – 225.9 h, June – 269.7 h), min–mean–max daily temperatures of 10–15.5–21°C in April, 12.6–17.5–22.6°C in May, and 17.9–22.5–27.6°C in June and were regularly watered with distilled water. The mean relative air humidity was 57, 63 and 66% in April, May, and June, respectively. During the rare rainy days/nights, the plant soil was protected from rain by an elastic plastic cover mounted on the pot, thinly wrapping the plant stem and being detached 15 cm from the soil surface. Otherwise, the pots were not covered. The experiment was carried out in five replicates per each treatment in a complete randomized block design.

The soil used in this study was collected from a depth of 15–20 cm at an experimental site of the INEP. The collected soil was air-dried and sieved (diameter <200 μm), and then placed in 10-L plastic pots (5 kg of soil per each pot). The soil samples were analysed for pH, organic carbon (C), total nitrogen (N), available forms of phosphorus (P_2O_5), and potassium (K_2O), and total content of different elements (Cd, Co, Cr, Cu, Fe, Mn, and Ni). For pH determination, 25 ml of double distilled water (pH_{H2O}) or 25 ml 1M KCl (pH_{KCl}) was added to 10 g of soil and stirred for 30 min, after which the pH value was measured directly in the suspension (*Iskra MA 5730*). Organic carbon was measured by the method of Tjurin (1965). Total nitrogen content was determined by Kjeldahl digestion (Bremner 1996), and available forms of phosphorus and potassium were analysed using the standard AL-method (Egner *et al.* 1960). The total element content in soil was determined according to method 3051 (USEPA 1998). The soil characteristics were as follows: pH_{H2O} 6.5; pH_{KCl} 5.4; 1.86% (organic C); 0.072% (total N);

18.34 mg(available P) $100g^{-1}$(DM); 17.97 mg (available K) $100g^{-1}$(DM); 0.14 mg(total Cd) kg^{-1}(DM); 7.45 mg (total Co) kg^{-1}(DM); 38.70 mg(total Cr) kg^{-1} (DM); 6.91 mg(total Cu) kg^{-1}(DM); 6614.0 mg(total Fe) kg^{-1}(DM); 777.3 mg(total Mn) kg^{-1}(DM); and 36.50 mg(total Ni) kg^{-1}(DM).

Quantification of Zn in soil and plants: The soils samples were dried at room temperature (25°C), sieved, and analysed for pH, total Zn concentration, and available Zn concentration for each treatment. Total Zn content in the soil was determined after digestion of sieved soil samples (< 200 µm) in HCl:HNO_3 (3:1, v/v) according to method USEPA 3051 (1998). In order to determine available Zn content in the soil, dried and sieved soil was mixed continuously for 2 h in 1 M ammonium acetate and 0.01 M EDTA mixture (pH 7), then filtered using *No. 40 Sartorius* filter papers according to Standard NF X 31-120 (Pansu and Gautheyroy 2006). The total and available Zn concentrations were determined by atomic absorption spectrophotometry (*Shimadzu AA-7000,* Japan). According to FAO (1983), the experimental soil can be classified as a luvic chernozem.

Plant parts (roots, rhizomes, stems, and leaves) were thoroughly washed in tap and deionized water. Air-dried plant material was ground with a ceramic mortar and pestle and then dried at 105°C to a constant mass. Plant material was digested in HNO_3:H_2O_2 (2:1, v/v) according to method USEPA 3052 (1996), after which the Zn content in plant parts was determined by atomic absorption spectrophotometry (*Shimadzu AA-7000*, Japan). For quality control, replicate samples, blanks, and standardized reference materials (Soil 90-0115-0106, Bureau Interprofessionnel d'Etudes Analytiques, BIPEA; apple leaves NIST 1515) were included in all analyses.

The phytostabilization potential of *M.* × *giganteus* for Zn was estimated from several factors: the bioconcentration factor (BCF), the bioaccumulation factor (BAF), and the translocation factor (TF), according to Baker (1981):

BCF = Zn concentration in roots/total Zn concentration in soil
BAF = Zn concentration in leaves/total Zn concentration in soil
TF = Zn concentration in leaves/Zn concentration in roots

Pigment extraction and quantification: The amounts of chlorophyll (Chl) *a,* Chl *b*, and total carotenoids (Car) in leaves were determined according to Hiscox and Israelstam (1979). Total Chl and Car were extracted with dimethyl sulfoxide (DMSO), and samples were centrifuged at 3,000 × *g* for 10 min. Absorption of Chl *a*, Chl *b*, and Car was determined by UV–Vis spectrophotometry (*Shimadzu UV-1800*, Japan) at 663, 645, and 480 nm, respectively. Chl and Car contents were calculated using equations according to Arnon (1949) and Wellburn (1994), their amounts expressed as mg per g of leaf DM.

Gas-exchange and Chl fluorescence measurements: All parameters were measured using an infrared gas analyser of the *CIRAS-2* type (*PP Systems*, USA) equipped with a Chl fluorescence module (CFM). Measurements of net photosynthetic rate (P_N), transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were performed on the penultimate well developed leaf. Conditions in the leaf chamber were as follows: leaf temperature 25°C, relative air humidity of 60%, 370 µmol(CO_2) mol^{-1}. Light-response curves (P_N/PPFD) were obtained at descending values of PPFD ranging from 2,000 to 0 µmol m^{-2} s^{-1}. The P_N as a function of increasing intercellular CO_2 concentrations (P_N responses to high C_i) was obtained at PPFD of 1,500 µmol m^{-2} s^{-1} and CO_2 concentrations of 370, 750, 1,000; 1,500; and 2,000 µmol(CO_2) mol^{-1} in the leaf chamber. Measurements were performed during the midmorning period under laboratory conditions at 21°C and low ambient irradiance.

For measurement of Chl *a* fluorescence, a leaf (predarkened for 30 min) was enclosed in a *PLC(U)* universal automatic cuvette (*PP-Systems*, USA) and the minimal level of fluorescence in the dark (F_0) was measured after 10 min of stabilization. Leaf was then exposed to a saturating pulse of actinic light [5,100 µmol(photon) m^{-2} s^{-1}, duration 700 ms] and the maximal fluorescence yield (F_m) was obtained. Values of F_0 and F_m were used to calculate the maximum quantum efficiency of PSII photochemistry (F_v/F_m).

Statistical analysis: All data are expressed as the mean ± standard deviation (M ± SD) of five replicates (n = 5). Group mean ranks were compared using the non-parametric *Mann-Whitney*´s *U* test with a significance level of $p<0.05$. Correlations between parameters were evaluated using *Spearman*'s rank correlation coefficient (ρ). All statistical analyses were performed in the *Statistica 7.0* software package (*StatSoft Inc*., Tulsa, USA, 2004).

Results

Concentrations of Zn in the soil and plant parts: Table 1 presents the total and available concentrations of Zn in treated soils (T_{250}, T_{500}, and $T_{1,000}$) in relation to the control soil and their respective pH values. The slightly lower pH values increased the pool of available Zn, which represents between 53 and 69% of the total Zn content in soils under the different Zn treatments (Table 1).

All plants grown in Zn-treated soils had significantly higher amounts of Zn in comparison with the control plants (Fig. 1*A*). The concentrations of Zn in roots, stems, and leaves largely corresponded to its amount in the associated soil, and were as follows: control plants (56.4, 56.0, and 15.1 mg kg^{-1}, respectively), T_{250} (337.2, 277.3, and 185.5 mg kg^{-1}, respectively), T_{500} (505.3, 528.2, and 177.3 mg kg^{-1}, respectively), and $T_{1,000}$ (704.0, 424.0, and 273.2 mg kg^{-1}, respectively) (Fig. 1*A*). Also, the concentrations of Zn in rhizomes of plants of the T_{250} (128.5 mg kg^{-1}), T_{500} (155.1 mg kg^{-1}), and $T_{1,000}$ (338.2 mg kg^{-1}) were significantly higher than in rhizomes of the control plants (26.0 mg kg^{-1}) (Fig. 1*A*).

The control plants showed statistically significantly lower amounts of Zn in all plant organs compared to all Zn-treated plants. Among Zn-treated plants, there were significant differences in the concentrations of Zn in the roots (between T_{250} and T_{500}, between T_{250} and $T_{1,000}$, and between T_{500} and $T_{1,000}$) and leaves (between T_{250} and $T_{1,000}$, and between T_{500} and $T_{1,000}$) (Fig. 1*A*). The bioconcentration factor (BCF) (0.70–1.11) and bioaccumulation factor (BAF) (0.23–0.61) for Zn were lower than 1 in all plants, except those of the T_{250}, where BCF slightly exceeded 1 (Fig. 1*B*). The translocation factor (TF) for Zn was lower than 1 (0.27–0.55) in all *M.* × *giganteus* plants grown in experimental soils (Fig. 1*B*). There were no statistically significant differences of BCF values between control and Zn-treated plants (Fig. 1*B*), whereas plants in $T_{1,000}$ had significantly lower BCF than in T_{250} and T_{500} (Fig. 1*B*). Plants in T_{250} showed higher BAF compared with the control, T_{500}, and $T_{1,000}$, whereas there were no statistically significant differences between control and plants in T_{500} and $T_{1,000}$ (Fig. 1*B*). In this study, T_{250} and $T_{1,000}$ plants had higher TF values than that in the control plants. At the same time, there were no significant differences of TF values between Zn-treated plants (between T_{250} and T_{500}, between T_{250} and $T_{1,000}$, and between T_{500} and $T_{1,000}$) (Fig. 1*B*).

Photosynthetic pigments: The amounts of Chl *a*, Chl *b*, Chl (*a*+*b*), and Car, together with values of the Chl *a/b* ratio are shown in Table 2. Differences in the content of Chl *a*, Chl (*a*+*b*), and Car between the control and Zn-treated plants were not statistically significant. However, significant differences of Chl *b* content were noted between control and T_{500} plants, and between control plants and those of the $T_{1,000}$. Furthermore, significant differences in values of the Chl *a*/*b* ratio were found between T_{250} and T_{500}, and between T_{250} and $T_{1,000}$.

After three months of treatment, Zn-treated plants showed visible signs of Zn toxicity in the form of chlorotic spots on the leaf lamina, observable only in mature leaves, and in $T_{1,000}$ plants even in the form of necrotic damage present along the leaf margins in the oldest leaves (Fig. 2*A*,*B*).

Table 1. The pH values and concentrations of total and available Zn in the control soil and soil treated with different concentrations of Zn. *Mann–Whitney U* test. Statistically significantly different values are separated by *different letters* for each column.

Treatment	pH_{H2O}	pH_{KCl}	Total Zn [mg kg^{-1}(DM)]	Available Zn [mg kg^{-1}(DM)]	Available pool of Zn in the total Zn [%]
Control	6.5	5.4	64 ± 9^a	9.3 ± 7.8^a	15
T_{250}	6.6	5.8	305 ± 1^b	161 ± 20^b	53
T_{500}	6.4	5.5	528 ± 28^c	366 ± 14^c	69
$T_{1,000}$	6.1	5.5	$1,015 \pm 82^d$	678 ± 23^d	67

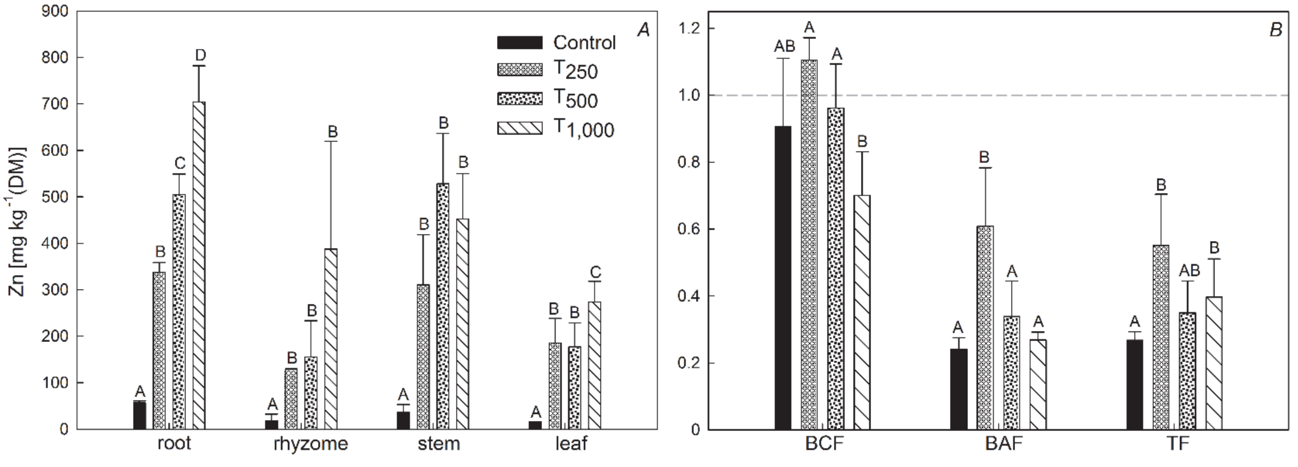

Fig. 1. Concentrations of Zn in different plant organs in *Miscanthus* × *giganteus* grown on different Zn concentrations (*A*); bioconcentration factor (BCF), bioaccumulation factor (BAF), and translocation factor (TF) for zinc (*B*). Statistically significantly different values are separated by *different letters* within each group of bars.

Table 2. Content of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and total carotenoids (Car) in leaves of *Miscanthus* × *giganteus* grown in the control soil and soils treated with different concentrations of Zn. *Mann–Whitney U* test. Statistically significantly different values are separated by *different letters* for each column.

Treatment	Chl (*a*+*b*) [mg g^{-1}(DM)]	Chl *a* [mg g^{-1}(DM)]	Chl *b* [mg g^{-1}(DM)]	Chl *a*/*b*	Car [mg g^{-1}(DM)]
Control	4.14 ± 1.70^{a}	3.08 ± 0.50^{a}	1.06 ± 0.15^{a}	2.91 ± 0.19^{a}	0.85 ± 0.19^{a}
T_{250}	4.09 ± 1.63^{a}	3.06 ± 0.65^{a}	1.03 ± 0.03^{a}	2.97 ± 0.39^{ab}	0.86 ± 0.59^{a}
T_{500}	3.53 ± 1.48^{a}	2.77 ± 0.28^{a}	0.76 ± 0.05^{b}	3.64 ± 0.28^{b}	0.80 ± 0.28^{a}
$T_{1,000}$	3.23 ± 1.28^{a}	2.52 ± 0.57^{a}	0.71 ± 0.11^{b}	3.55 ± 0.28^{b}	0.71 ± 0.28^{a}

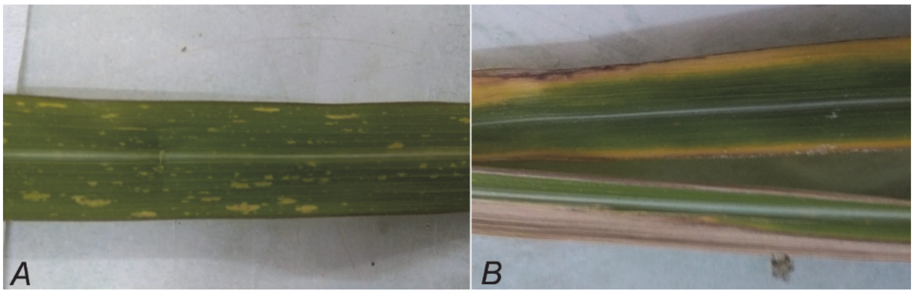

Fig. 2. Visible damage on leaves caused by Zn excess: chlorotic spots on mature leaf (*A*) and dry margins on old leaves (*B*).

Gas exchange and Chl fluorescence: Although in all plants P_N was saturated at a relatively high PPFD of 2,000 μmol m^{-2} s^{-1}, all plants treated with a Zn surplus showed almost two-fold lower photosynthetic activity at maximum PPFD compared to the control plants (Fig. 3*A*). Increase in C_i under constant light intensity (PPFD of 1,500 μmol m^{-2} s^{-1}) resulted in a uniform increase of P_N in both the control and all Zn-treated plants, with photosynthetic activity decreasing in proportion to increase in the concentration of applied Zn (Fig. 3*B*).

The parameters P_N, *E*, g_s, and C_i were significantly lower in all Zn-treated plants compared to the control ones (Table 3). Respiration (*R*) was significantly higher in the control and T_{250} than that in plants treated with higher concentrations of Zn (T_{500}, $T_{1,000}$). Fluorescence parameters F_m, F_v, and F_v/F_m were significantly lower, whereas F_0 was significantly higher in all Zn-treated plants than in the control. In addition, F_m, F_v and F_v/F_m were significantly higher in T_{250} and T_{500} in comparison with $T_{1,000}$.

Negative correlations were found between the amount of Zn in leaves and P_N, *E*, g_s, C_i, fluorescence parameters (F_m, F_v, F_v/F_m), and Chl *b* content. Positive correlations were found between the concentration of Zn in leaves and both *R* and the Chl *a/b* ratio (Table 4). Furthermore, significant correlations were found between parameters of gas exchange, Chl *a* fluorescence, and pigment content (Table 4).

Discussion

Frequent Zn contamination of surface soils originating from prolonged use of Zn fertilizers, input from industrial pollution, and mining activities leads to Zn toxicity in plants (Kabata-Pendias 2011, Martens and Smolders 2013). Plants differ in their ability to tolerate elevated concentrations of Zn in the soil, exhibiting different strategies of coping with excessive amounts of Zn that are involved in its absorption, translocation, and redistribution within the plant (Sinclair and Krämer 2012, Tsonev and Lidon 2012).

The concentrations of Zn applied to the soil in all treatments were higher than the mean amounts of Zn present in different undisturbed and agricultural soils in the world (Eriksson 2001, Takeda *et al.* 2004, Herselman *et al.* 2005, Kabata-Pendias 2011). The concentrations of soluble Zn in treated soils ranged from 161 to 366 and 678 mg kg^{-1}(DM), which were 17.2, 39.2, and 72.6 times higher than its concentration in the unpolluted control soil. As a consequence, the amounts of Zn in all Zn-treated plants were significantly higher than in the control ones, and were on concentrations considered toxic to most plant species (Macnicol and Beckett 1985, Kabata-Pendias 2011). The strong positive correlation between Zn in plant roots and Zn in the soil indicates that the amounts of Zn in roots strongly depend on its concentration in the soil. However, the progressive decrease of BCF with increasing Zn concentration in the soil indicated that *M.* × *giganteus* limited absorption of Zn to roots at higher Zn concentrations in the soil. Furthermore, according to relatively low BAF and TF values and the mostly uniform concentration of Zn in leaves between treatments, the Zn translocation from roots to leaves was restricted. The

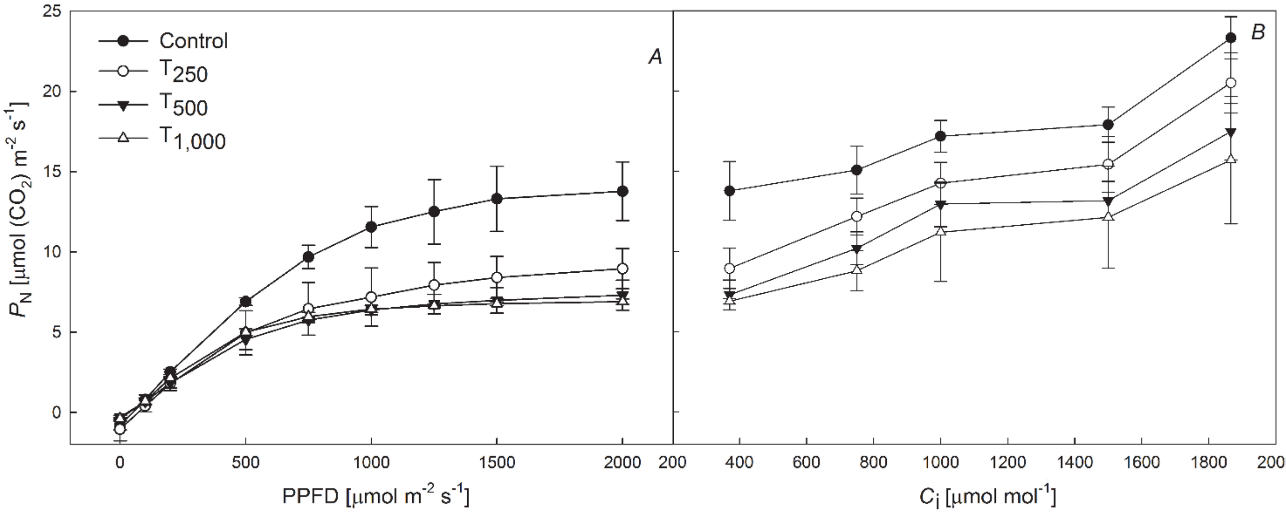

Fig. 3. Photosynthetic rate (P_N) in *Miscanthus* × *giganteus* grown on different Zn concentrations as a function of photosynthetic photon flux density (PPFD) (light-response curves, P_N/PPFD) (*A*) and of increasing intercellular CO$_2$ concentrations (C_i) (P_N responses to high C_i, *B*).

Table 3. Parameters of gas exchange and Chl fluorescence in *Miscanthus* × *giganteus* grown in the control soil and soils treated with different concentrations of Zn (P_N – net photosynthetic rate; *R* – respiration; *E* – transpiration rate; g_s – stomatal conductance; C_i – leaf intercellular CO$_2$ concentration, F$_0$ – minimal fluorescence yield of the dark-adapted state; F$_m$ – maximal fluorescence yield of the dark-adapted state; F$_v$ – variable fluorescence; F$_v$/F$_m$ maximal quantum yield of PSII photochemistry). *Mann–Whitney U* test. Statistically significantly different values are separated by *different letters* for each column.

Parameter	Control	T_{250}	T_{500}	$T_{1,000}$
P_N [μmol m^{-2}s^{-1}]	11.1 ± 1.18^a	6.8 ± 2.02^b	6.4 ± 0.36^b	6.4 ± 0.21^b
R [μmol(CO$_2$) m^{-2} s^{-1}]	–1.2 ± 0.00^a	–1.3 ± 0.62^a	–0.33 ± 0.11^b	–0.40 ± 0.10^b
E [mmol(H$_2$O) m^{-2} s^{-1}]	1.7 ± 0.30^a	1.06 ± 0.25^b	0.97 ± 0.29^b	0.87 ± 0.58^b
g_s [mmol(H$_2$O) m^{-2} s^{-1}]	101.0 ± 19.44^a	53.0 ± 13.75^b	50.3 ± 12.86^b	45.0 ± 3.6^b
C_i [μmol(CO$_2$) mol^{-1}]	148.0 ± 0.00^a	143.7 ± 14.57^a	141.7 ± 37.58^a	112.0 ± 22.07^b
F$_0$	498.6 ± 30.00^a	562.0 ± 4.58^b	517.7 ± 36.0^a	534.3 ± 60.54^{ab}
F$_m$	2386.3 ± 143.51^a	2091.7 ± 52.59^b	2043.3 ± 137.71^{bc}	1854.7 ± 170.88^c
F$_v$	1887.6 ± 122.68^a	1529.7 ± 55.08^b	1525.7 ± 103.5^{bc}	1317.3 ± 219.15^c
F$_v$/F$_m$	0.790 ± 0.01^a	0.736 ± 0.01^b	0.746 ± 0.00^b	0.704 ± 0.06^c

concentrations of Zn in the rhizome were 2–3 times lower than that in the roots, indicating that most of the Zn was retained within the roots or was translocated to the stem. On the basis of the concentrations of Zn in its organs, it can be asserted that *M.* × *giganteus* represents an excluder plant species suitable for Zn phytostabilization. This is in accordance with previously reported low accumulation of Zn, Cu, Ni, Cd, Pb, As, and Sb in aerial biomass of *M.* × *giganteus* (Fernando and Oliviera 2004, Wanat *et al.* 2013, Korzeniowska and Stanislawska-Glubiak 2015, Kocoń and Jurga 2017).

Although the Zn concentrations detected in leaves were in the range considered as toxic to most plant species (Macnicol and Beckett 1985, Kabata-Pendias 2011), signs of Zn toxicity were not visible in young leaves, but were observed only in the oldest leaves in the form of small chlorotic areas on the leaf lamina and necrotic leaf margins. The detected leaf damage indicates that over time Zn was concentrated in older leaves, where it induced their senescence, as it was previously shown in several different plant species (Prasad 2004).

Regarding pigments, high contents of Zn in *M.* × *giganteus* leaves resulted in the decline of Chl *b* content (ρ = –0.5594) and a consequent increase of the Chl *a/b* ratio (ρ = 0.6014), pointing to higher sensitivity of Chl *b* to Zn stress compared to Chl *a* and Car. Similarly, Li *et al.* (2014) observed that the amount of Chl (*a*+*b*) in leaves of *Miscanthus sacchariflorus* and *Arundo donax* did not change significantly under the influence of different concentrations of Zn in the soil [250, 500, 1,000; and 2,000 mg kg^{-1}(DM)]. Since Chl *b* builds the light-harvesting protein complex (LHCP) (Lambers *et al.* 1998), decreased content of Chl *b* and high values of the Chl *a/b* ratio in Zn-stressed *M.* × *giganteus* leaves indicate sensitivity of the LHCP. However, the insignificant changes in the content of Chl *a* and Car in Zn-stressed plants suggest low sensitivity of photochemical reaction centres and high efficiency of Car in protecting the photosynthetic system from photoinhibition (Farage *et al.* 2006).

At maximum light intensity of 2,000 μmol(photon) m^{-2} s^{-1}, the control plants had 2–3 times higher rates of photosynthesis than the Zn-treated plants, confirming that P_N is highly sensitive to elevated Zn concentrations. The presence of excessive Zn negatively affected all measured parameters of leaf gas exchange; it caused decrease of E (ρ = –0.6801), g_s (ρ = –0.6725), C_i (ρ = –0.7254), and P_N (ρ = –0.5814). Similarly, decrease of both P_N and g_s were reported in *M.* × *giganteus* leaves exposed to As, Pb, and Sb (Wanat *et al.* 2013) and in three different *Miscanthus* species exposed to Cd (Guo *et al.* 2016). In Zn-stressed plants, reduced values of g_s decreased C_i (ρ = 0.4797) and P_N (ρ = 0.9263), indicating the existence of stomatal limitation of photosynthesis. Furthermore, Sagardoy *et al.* (2009, 2010) found that stomatal and mesophyll conductances to CO_2 are the main limitations to photosynthesis in sugar beet (*Beta vulgaris*) plants grown in the presence of excess Zn. This negative effect of elevated Zn on g_s can be attributed to its influence on stomatal movement through the inhibition of water channels, as shown by Yang *et al.* (2004), and to reduction of K^+ uptake (Tsalandzono *et al.* 1994, Vaillant *et al.* 2005). According to Van Assche and Clijsters (1986), high Zn concentrations can also decrease Rubisco activity, since Zn can replace Mg in the enzyme's structure.

The values of F_v/F_m detected in the control plants were in the range that is considered optimal for plants (0.750–0.850) (Björkman and Demmig 1987). In T_{250} and T_{500} plants its values were at the lower optimum level, while those of the $T_{1,000}$ plants it was slightly below the optimal range. Decline in values of F_v/F_m pointed to the photoinhibiton of PSII and indicated a moderate negative impact of Zn concentrations on photosynthetic activity in *M.* × *giganteus*. The presented findings are in accordance with results obtained on *M.* × *giganteus* treated with high concentrations of As, Pb, and Sb (Wanat *et al.* 2013), and *Lolium perenne* (Bonnet *et al.* 2000), *Pisum sativum* (Szalontai *et al.* 1999), and *Datura* sp. (Vaillant *et al.* 2005) treated with excess Zn. In the present study, changes in the F_v/F_m ratio in Zn-stressed plants (ρ = –0.5804) occurred due to simultaneous increase in F_0 (ρ = –0.7762), decrease in F_m (ρ = 0.6014), and decrease in F_v (ρ = 0.6294), which is consistent with results previously obtained on the aforementioned plant species (Szalontai *et al.* 1999, Vaillant *et al.* 2005) and for *M.* × *giganteus* grown on soil contaminated with Zn, Pb, and Cd (Pogrzeba *et al.* 2017). The decrease of F_m may indicate that the Q_A reduction rate is high and electron transfer from quinone acceptors (Q_A) to the next carrier (Q_B) is slowed (Baker and Rosenqvist 2004). The smaller amount of Chl *b* in Zn-treated *M.* × *giganteus* suggests dissociation of the LHCPII complex from the PSII core, which can be linked with the observed low F_m (ρ = 0.7552) and F_v (ρ = 0.7063) values. Similar results were obtained by other authors, who

Table 4. *Spearman*'s rank order correlation coefficients for the photosynthetic rate (P_N), respiration in dark (R), transpiration (E), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), maximum quantum efficiency of PSII photochemistry (F_v/F_m), minimal level of fluorescence (F_0); maximal level of fluorescence (F_m); variable fluorescence (F_v), concentration of Chl *a*, concentration of Chl *b*, concentration of Chl (*a*+*b*), the Chl *a/b* ratio, and concentration of total carotenoids (Car) in leaves of *Miscanthus* × *giganteus* grown with different Zn concentrations.

Parameter	Leaf Zn	P_N	R	E	g_s	C_i	F_v/F_m	F_0	F_m	F_v	Chl *a*	Chl *b*	Chl (*a*+*b*)	Chl *a/b*	Car
Leaf Zn	1.0000	–0.5814	0.5887	–0.6801	–0.6725	–0.7254	–0.5804	0.1678	–0.7972	–0.7902	–0.2028	–0.5594	–0.2308	0.6014	–0.2378
P_N	–0.5814	1.0000	–0.5045	0.8868	0.9263	0.3280	0.5954	–0.2767	0.8091	0.8336	0.0876	0.4974	0.1366	–0.7356	0.0701
R	0.5887	–0.5045	1.0000	–0.5475	–0.4760	–0.3143	–0.2341	–0.0780	–0.5249	–0.4965	–0.2128	–0.7164	–0.3334	0.7873	–0.2624
E	–0.6801	0.8868	–0.5475	1.0000	0.9300	0.5436	0.6154	–0.1403	0.7989	0.7989	0.0576	0.4642	0.0576	–0.8205	0.0504
g_s	–0.6725	0.9263	–0.4760	0.9300	1.0000	0.4797	0.406305	–0.0840	0.6269	0.65499	0.0525	0.39930	0.0420	–0.6584	0.0420
C_i	–0.7254	0.3280	–0.3143	0.5436	0.4797	1.0000	0.1549	0.3380	0.7042	0.6549	0.4225	0.5211	0.3662	–0.3803	0.4225
F_v/F_m	–0.5804	0.5954	–0.2341	0.6154	0.406305	0.1549	1.0000	–0.7762	0.6014	0.6294	0.1608	0.3007	0.1678	–0.4406	0.1958
F_0	0.1678	–0.2767	–0.0780	–0.1403	–0.0840	0.3380	–0.7762	1.0000	–0.1189	–0.1608	0.0629	0.0350	0.0070	0.0979	0.0769
F_m	–0.7972	0.8091	–0.5249	0.7989	0.6269	0.7042	0.6014	–0.1189	1.0000	0.9930	0.4685	0.7552	0.4895	–0.6573	0.4895
F_v	–0.7902	0.8336	–0.4965	0.7989	0.65499	0.6549	0.6294	–0.1608	0.9930	1.0000	0.4266	0.7063	0.4475	–0.6294	0.4545
Chl *a*	–0.2028	0.0876	–0.2128	0.0576	0.0525	0.4225	0.1608	0.0629	0.4685	0.4266	1.0000	0.4202	0.9720	0.0420	0.9650
Chl *b*	–0.5594	0.4974	–0.7164	0.4642	0.39930	0.5211	0.3007	0.0350	0.7552	0.7063	0.4203	1.0000	0.4112	–0.5944	0.4133
Chl (*a*+*b*)	–0.2308	0.1366	–0.3334	0.0576	0.0420	0.3662	0.1678	0.0070	0.4895	0.4475	0.9720	0.4112	1.0000	–0.0699	0.9510
Chl *a/b*	0.6014	–0.7356	0.7873	–0.8205	–0.6584	–0.3803	–0.4406	0.0979	–0.6573	–0.6294	0.0419	–0.5944	–0.0699	1.0000	0.0070
Car	–0.2377	0.0701	–0.2624	0.0504	0.0420	0.4225	0.1958	0.0769	0.4895	0.4545	0.9650	0.4133	0.9510	0.0070	1.0000

recorded a small decrease of F_v/F_m in the presence of a Zn surplus in plants (Dhir *et al.* 2008, Sagardoy *et al.* 2010). In the present study, positive correlations of Chl *a* content and Chl (*a*+*b*) content with the value of F_m ($\rho = 0.4685$ and $\rho = 0.4895$, respectively) and of Car content with Chl *a* content ($\rho = 0.9650$), Chl (*a*+*b*) content ($\rho = 0.9510$), the value of F_m ($\rho = 0.4895$), and that of F_v ($\rho = 0.4545$) pointed to good ability of *M.* × *giganteus* to maintain high photosynthetic efficiency under conditions of Zn excess.

The moderate decrease of F_v/F_m in Zn-stressed *M.* × *giganteus* indicates: (*1*) the presence of specific mechanisms for effective immobilization of Zn within leaves; and (*2*) high efficiency of Car and a number of antioxidant enzymes in protecting the photosynthetic system from oxidative destruction (Bonnet *et al.* 2000, Sun *et al.* 2005, Farage *et al.* 2006, Sagardoy *et al.* 2010, Lu 2011, Nadgórska-Socha *et al.* 2013).

Conclusions: The concentrations of Zn in *M.* × *giganteus* plants followed those of total and available Zn in the associated soils, indicating that the plant was unable to prevent uptake of Zn, but accumulated it in varying amounts in different plant organs. However, the plants restricted their uptake of Zn, especially at higher Zn concentrations in the soil, which resulted in lower BCF, BAF, and TF values in the presence of higher amounts of applied Zn. Considering that only the concentrations of Zn in the underground organs of the plants reflected external Zn concentrations, whereas the Zn concentration in their aboveground organs remained constant over the experimental range of soil concentrations, we can conclude that *M.* × *giganteus* is a Zn excluder plant species. Excess Zn induced visible leaf damage in the form of chlorosis and necrosis only in the oldest leaves, indicating Zn accumulation over time. Despite a moderate negative effect of Zn surplus on gas exchange and stomatal conductance limitation of photosynthesis, *M.* × *giganteus* exhibited a high photosynthetic capacity in the presence of several excessive concentrations of Zn, indicating its high Zn tolerance and ability to grow on Zn-contaminated soils.

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