Chlorophyll Fluorescence as a Criterion for the Diagnosis of Abiotic Environmental Stress of *Miscanthus x Giganteus* Hybrid

Ján Tomaškin¹, Judita Tomaškinová²*, Hubert Theuma³

¹Department of Environment, Faculty of Natural Sciences, Matej Bel University, Tajovského 55, 974 01 Banská Bystrica, Slovak Republic
²Institute of Applied Sciences, Malta College of Arts, Science & Technology, Corradino Hill, Paola PLA 9032, Malta
³Nature Trust-FEE Malta, Wied Ghollieqa Environment Centre, Lower Level, Car Park 1, University of Malta, Msida, Malta

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Abstract

This study aimed to assess the impact of abiotic chemical stresses in the soil environment (salinization, acidification, inorganic risk elements from the industry) on the chlorophyll content and parameters of chlorophyll fluorescence - effective and maximum quantum yield PSII (yield Y(II) or Fv/Fm test) of *Miscanthus x giganteus* hybrid. For this objective, graduated doses of stresses, as mentioned above, were applied to a pot experiment’s controlled conditions during the growing season 2018. The chlorophyll content of 19.267 CCI units was recorded with the control treatment. However, a significantly higher content of chlorophyll was observed with the examined stressors, especially salinity (25.433 CCI) and acidity (26.500 CCI). Y (II) value ranged from 0.561 to 0.693 and Fv/Fm from 0.689 to 0.775 respectively. There were no significant differences between assessed stressors and control. *Miscanthus x giganteus* had good resistance to the impact of assessed chemical stresses; damage to the assimilation apparatus of the plants did not occur. The species has the potential and can create the preconditions for cultivation even in a mildly contaminated soil environment, e.g., for reclamation of degraded soils or energy use.

Keywords: effective quantum yield PSII, environmental stress, Fv/Fm test, chemical stressor, chlorophyll fluorescence; maximum quantum yield PSII, *Miscanthus x giganteus*, soil acidification, soil salinization, yield Y (II)

*e-mail: tomaskinova@mcast.edu.mt*
Introduction

One of the present environmental problems is soil degradation and contamination [1].

Kertész [2] report that degraded land occupies an area of 323 million hectares (3.7% of total land used), with a chemically degraded area of 240 million hectares (loss of nutrients 136, salinization 77, pollution 21 and acidification 6 million hectares) and a physically degraded area of 83 million hectares. The mitigation of potential health hazards and land scarcity due to land-use change can be addressed by restoring the function and ecosystem services of contaminated land. Physico-chemical remediation options are criticized as being costly and not providing environment-friendly solutions. Nature-based solutions based on the use of plants and associated microorganisms could be a sustainable, cost-effective option to reduce pollutant exposure. Phytomanagement aims at using valuable non-food crops to alleviate environmental and health risks induced by pollutants, and at restoring ecosystem services. Suitable plant species must be tolerant to contaminants, reduce their transfer into the food chain, and efficiently produce marketable biomass [3].

In this study, the impact of chemical soil degradation, especially salinization, acidification, and industrial pollution by inorganic elements on vegetation, was investigated. These factors were assessed under controlled conditions in a pot experiment where Miscanthus x giganteus J.M. Greef et Deuter. (hybrid of Miscanthus sinensis and Miscanthus sacchariflorus) was selected as a model plant taxon. Miscanthus x giganteus is considered as a multi-use crop [4]. This taxon has been chosen because it is a non-invasive hybrid and has very often been tested for its reaction to abiotic chemical factors; because of its tolerance to polluted soils, it is recommended for phytoremediation of contaminated soils [5-7].

Korzeniowska, Stanisławska-Glubiak [8] evaluated of Miscanthus x giganteus and Spartina pectinata suitability phytoremediation of Cu, Ni, and Zn. Their results confirmed the higher resistance of Spartina pectinata to Cu, Ni and Zn soil contamination compared with Miscanthus x giganteus. Miscanthus x giganteus is a perennial C4 grass, which also shows great potential as a crop with a high yield of biomass [9]. It exhibits high dry matter yields (8-32 t DM (dry mass)/ha per year) depending on environmental conditions, and these properties indicate Miscanthus x giganteus as one of the more promising energy crops [10, 11].

Under favourable growing conditions and sufficient soil moisture, Miscanthus x giganteus may grow to 25 t ha\(^{-1}\) of dry material. Also, grass may accumulate 10-20 t ha\(^{-1}\) of rhizome mass. As the plant harvest mass increases, additional CO\(_2\) is taken from the atmosphere by Miscanthus x giganteus. Thus, these plants reduce atmospheric carbon dioxide to mitigate greenhouse effects [12]. Miscanthus x giganteus is one of the perennial grasses that has been identified as one of the best low-input bioenergy production options [13].

Salinization, acidification, and industrial pollution represent abiotic chemical stressors, which often have a negative impact on the health state of vegetation, and ultimately, the death of individual plants or the entire population may occur. Mesi and Kopliku [14] reported that plants exposed to an excess of metals might undergo alterations in a wide spectrum of physiological features. The influence of stressors on plants can be characterized as a stress response, which is manifested by anatomical- morphological and functional changes [15]. The first manifestation of stress on plants or their leaves is damage to the assimilation apparatus, especially the photosystem II (PSII) [16]. The measurement of chlorophyll fluorescence provides information on the extent to which PSII can absorb and utilize photosynthetically active radiation. The content of chlorophyll in leaves and parameters of chlorophyll fluorescence can be used as indicators of stress induced by abiotic chemical stressors [17, 18]. Several studies from Slovakia also confirmed that abiotic environmental factors caused changes in the chlorophyll content of plant leaves [19-22].

The objective of this study was to assess the impact of selected abiotic chemical stressors on the content and parameters of chlorophyll fluorescence and the vitality of Miscanthus x giganteus hybrid.

Material and Methods

During the growing season 2018, a pot experiment was conducted to assess the impact of salinity, acidification and industrial pollution on the chlorophyll content and fluorescence parameters of Miscanthus x giganteus hybrid (further M. x giganteus) (at the Department of Environment, Faculty of Natural Sciences, Matej Bel University, Banská Bystrica, Slovakia, GPS: 48° 74' N, 19° 12’ E). The pot experiment was under a shelter that let in natural sunlight, but the plants were protected from rain. The plants had controlled irrigation at a dose of 1 dm\(^3\) of drinking water every 4 days throughout the experiment. The average annual temperature was 10.0°C; during the growing season (April to September) it was 17.6°C and during the experiment it was 18.9°C (June), 20.1°C (July), 21.0°C (August) and 15.1°C (September).

The trial consisted of three graduated levels of salinity, acidity, and inorganic risk elements from the magnesite and aluminium industry as well. M. x giganteus plants were obtained from the Gene bank of the Slovak Republic (NAFC-PPRI Piešťany). On 14th June 2018, two plants were planted in each pot. Large-volume pots were filled with 8.5 dm\(^3\), 9.0 dm\(^3\), 9.5 dm\(^3\), and 10.0 dm\(^3\) of a gardening substrate, respectively (Table 1). The salinity and acidity of the soil environment were simulated by applying saline...
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Inorganic pollution was represented by outlets from the area of the magnesite industry at a site within in the city of Jelšava (48°38’N, 20°13’E, in the eastern part of Central Slovakia), and the red mud generated during the production process of alumina from bauxite ore at a site within the city of Žiar nad Hronom (48°33’N, 18°50’E, in the western part of Central Slovakia). Samples of contaminated soil (originating from the vicinity of the magnesite plant SMZ a.s. Jelšava) and red mud (originating from the vicinity of the alumina plant Slovalco a.s. at Žiar nad Hronom) - of an amount of 1, 2 and 3 kg were mixed with the gardening substrate (producer: AGRO CS a.s. based in the Czech Republic. The manufacturer states the following chemical and physical properties of the substrate: pH value 6.5-7.0, content of total N as N max. 1.9%, content of total P as P₂O₅ max. 0.5%, content total K as K₂O max. 0.9%, particles over 20 mm max. 5%, humidity max. 65%, content of combustible substances min. 50%, electrical conductivity max. 1.2 mS.cm⁻¹). Control treatment included only 10 dm³ of gardening substrate. Experimental treatments, including the control treatment, are shown in Table 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Abiotic chemical stressor</th>
<th>Dose of chemical stressor</th>
<th>Volume of gardening substrate (in dm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Salinity</td>
<td>20 g NaCl/2 dm³ H₂O = 10‰</td>
<td>10.0</td>
</tr>
<tr>
<td>A2</td>
<td>Salinity</td>
<td>30 g NaCl/2 dm³ H₂O = 15‰</td>
<td>10.0</td>
</tr>
<tr>
<td>A3</td>
<td>Salinity</td>
<td>60 g NaCl/2 dm³ H₂O = 30‰</td>
<td>10.0</td>
</tr>
<tr>
<td>B1</td>
<td>Acidity</td>
<td>pH 6</td>
<td>10.0</td>
</tr>
<tr>
<td>B2</td>
<td>Acidity</td>
<td>pH 5</td>
<td>10.0</td>
</tr>
<tr>
<td>B3</td>
<td>Acidity</td>
<td>pH 4</td>
<td>10.0</td>
</tr>
<tr>
<td>C1</td>
<td>Solid pollutants (MgO)</td>
<td>1 kg</td>
<td>9.5</td>
</tr>
<tr>
<td>C2</td>
<td>Solid pollutants (MgO)</td>
<td>2 kg</td>
<td>9.0</td>
</tr>
<tr>
<td>C3</td>
<td>Solid pollutants (MgO)</td>
<td>3 kg</td>
<td>8.5</td>
</tr>
<tr>
<td>D1</td>
<td>Red mud</td>
<td>1 kg</td>
<td>9.5</td>
</tr>
<tr>
<td>D2</td>
<td>Red mud</td>
<td>2 kg</td>
<td>9.0</td>
</tr>
<tr>
<td>D3</td>
<td>Red mud</td>
<td>3 kg</td>
<td>8.5</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>10.0</td>
</tr>
</tbody>
</table>

Table 1. Treatments utilized in experiment.

During the growing season 2018, three measurements of chlorophyll content and Chlorophyll fluorescence were taken on 4th August 2018, 19th August 2018, and 3rd September 2018.

Chlorophyll content (CCI - chlorophyll content index) in fully expanded leaves was measured with a Chlorophyll Content Meter CCM-200 plus (Opti-Sciences. Inc., USA). Higher CCI values indicated a higher chlorophyll concentration. Chlorophyll fluorescence was evaluated by tests Yield Y (II) and Fv/Fm with a chlorophyll fluorometer OS5p (Opti-Sciences, Inc., USA). These tests are standard stress tests used to identify and quantify the level of stress in plants. The result of the measurements was the effective quantum yield PSII - Y (II), which was measured during daylight and the maximum quantum yield PSII (Fv/Fm), which was measured 30 minutes after leaf-shading. For most plant species, the optimal values of Fv/Fm range from 0.79 to 0.84, reduced values indicate plant stress [16]. Also, Malinská et al. [25] report that measuring leaf fluorescence can be a useful tool for detecting plant stress in vivo. They assessed the Fv/Fm parameter and the performance index on M. x giganteus leaves and considered these parameters to be a suitable method for identifying significant plant stress, but they add that other indexes may detect some minor changes.

Dissipation and trapping flux per reaction centre and electron transport to PSI electron acceptors, seem to be highly sensitive markers for detecting the slight differences in plant photochemistry.

Chlorophyll content and chlorophyll fluorescence were subjected to a two-way ANOVA (LSD α 0.05). Statistical analysis was carried out with Statgraphics software version 5.0.
Results and Discussion

The impact of different doses of abiotic chemical stressors and date of three measurements on changes of values of quantum yield PSII and chlorophyll content were assessed. The mean values of quantum yield PSII, chlorophyll content, and statistical analysis results are shown in Table 2.

The values of effective quantum yield PSII Y(II) varied from 0.561 (Treatment B1) to 0.693 (Treatment B2). No significant differences within the treatments were found, and we can conclude that the evaluated abiotic stressors did not affect the variability of this trait.

Similarly to different doses of abiotic stressors, significant differences were not recorded for dates of measurement.

By contrast, maximum quantum yield PSII Fv/Fm showed a more sensitive response to abiotic stressors. During growing season, the lowest value (0.689) was recorded with treatment C3 and the highest values (0.767, 0.775) with treatments B1 and D1, respectively. While there were significant differences between these treatments, no significant differences were found between the other treatments, including control. Similarly to effective quantum yield PSII Y, no significant differences were recorded for dates of measurement. M. x giganteus displayed relatively good tolerance to the abiotic environmental load, and its quantum yields PSII did not show any significant destruction. M. x giganteus was tolerant to a lower salinity level (A1) of soil environment (Fv/Fm = 0.763); there was a decrease in Fv/Fm (0.734) with higher soil salinity (A3) which indicates mild plant stress. More specifically, the reaction of M. x giganteus to the salinity of the soil was dealt with by Plazek et al. [26] state that M. x giganteus is a species that is useful for sustainable agriculture and can be cultivated in soil with even higher salinity. They evaluated the impact of soil salinity (0 - control, 100, and 150 mM NaCl) on chlorophyll fluorescence (Fv/Fm). A significant difference in the maximum quantum yield of PSII (Fv/Fm) was not detected in the control with saline of 100 mM NaCl.

Elevated soil salinity of 150 mM NaCl has significantly reduced the maximum quantum yield of PSII (Fv/Fm). Changes in chlorophyll fluorescence (as well as net photosynthesis rate, fresh and dry weight of roots and leaves, and potassium and proline concentrations in leaves) are the most reliable indicators.

Table 2. The impact of environmental stressors on quantum yields PSII and chlorophyll content of Miscanthus x giganteus hybrid.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Quantum yields PSII</th>
<th>Chlorophyll content (CCI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Y(II)</td>
<td>Fv/Fm</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>0.662 a</td>
<td>0.763 b</td>
</tr>
<tr>
<td>A2</td>
<td>0.676 a</td>
<td>0.753 ab</td>
</tr>
<tr>
<td>A3</td>
<td>0.563 a</td>
<td>0.734 ab</td>
</tr>
<tr>
<td>B1</td>
<td>0.561 a</td>
<td>0.767 b</td>
</tr>
<tr>
<td>B2</td>
<td>0.693 a</td>
<td>0.718 ab</td>
</tr>
<tr>
<td>B3</td>
<td>0.675 a</td>
<td>0.753 ab</td>
</tr>
<tr>
<td>C1</td>
<td>0.683 a</td>
<td>0.763 b</td>
</tr>
<tr>
<td>C2</td>
<td>0.662 a</td>
<td>0.694 ab</td>
</tr>
<tr>
<td>C3</td>
<td>0.648 a</td>
<td>0.689 a</td>
</tr>
<tr>
<td>D1</td>
<td>0.652 a</td>
<td>0.775 b</td>
</tr>
<tr>
<td>D2</td>
<td>0.622 a</td>
<td>0.759 b</td>
</tr>
<tr>
<td>D3</td>
<td>0.623 a</td>
<td>0.753 ab</td>
</tr>
<tr>
<td>Control</td>
<td>0.675 a</td>
<td>0.764 b</td>
</tr>
<tr>
<td>LSD α_{0.05}</td>
<td>0.14144</td>
<td>0.06590</td>
</tr>
<tr>
<td>Measurement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.674 a</td>
<td>0.737 a</td>
</tr>
<tr>
<td>2</td>
<td>0.619 a</td>
<td>0.758 a</td>
</tr>
<tr>
<td>3</td>
<td>0.644 a</td>
<td>0.753 a</td>
</tr>
<tr>
<td>LSD α_{0.05}</td>
<td>0.06795</td>
<td>0.03166</td>
</tr>
</tbody>
</table>

Statistic method: Multifactor ANOVA - 95.0 % LSD test (α = 0.05). The values in the same column with different letters a, b, c, d, e, f, g are significantly different at P<0.05 level.
of Miscanthus' response to salinity. They further state that soil salinity of 100 and 150 mM NaCl did not damage membrane structures in leaves. The results show that M. x giganteus tolerates slightly elevated salinity of the soil environment.

Stavridou et al. [27] reported that higher concentrations and longer-lasting salinity affected the decrease in Fv/Fm in their experiment, which essentially indicates the response of plants to stress. Similarly, Hsu [28] states that Miscanthus may be grown on soils with elevated salinity. This fact is documented by the results in our research (assessment).

The highest doses of other evaluated stressors (B3, C3, D3) gradually reduced the values of the Fv/Fm test, but apart from C3 (0.689), it was not a statistically significant reduction compared to the control (0.764). In connection with acidification, Strasil [10] indicated that optimal soil pH for M. x giganteus ranges from 5.5 to 6.5. On the contrary, yield depressions were observed from 5.5 to 7.5 are recommended, and weak growth has occurred on soils having an alkaline pH of 8 and above.

Kayama [31] summarized pH data from the topsoil (0-10 cm) of growth habitats of Miscanthus sinensis (parent species for M. x giganteus) in Japan and noted that, although plants were able to grow in soils with a pH range of 3.5 to 7.5, most were found to be growing in soils within a pH range between 4.0 and 6.0. Some Miscanthus species such as Miscanthus sinensis (further M. sinensis) grow on neutral or acid-sulfate soils (pH = 4-6) that often have relatively high Al levels [32].

An et al. [33] described the pH of soils in Rankoshi, Hokkaido, Japan (100-200 m above sea level), which Miscanthus had colonized as ranging from 2.7 to 5.4, but pH values between 3.5 and 3.9 were more often observed. These pH ranges may, however, only be applicable to particular genotypes since M. sinensis seems to be tolerant to a broad range of soil pH values [31].

The results of the stress impact of solid pollutants and red mud on quantum yields PSII (Fv/Fm test) do not appear in the scientific literature; rather, it is often stated based on biomass production that M. x giganteus can grow on highly polluted substrates, and in fact, it often features as a short-term soil phytostabilizer without any inputs [34].

Similarly, also Figala et al. [6] and Techer et al. [5] monitored the impact of industrial inorganic pollution (e.g. heavy metals) on the vitality of the species under our assessment.

They state that Miscanthus tolerates a mildly contaminated soil environment. Similar conclusions were formulated by Arduini et al. [35], that Miscanthus as a non-forage plant is planted to eliminate impacts of industrial waste areas contaminated with heavy metal ions.

Accordingly, Fernando, and Oliveira [36] indicated that besides the high biomass yield, M. x giganteus is a metal-tolerant plant that does not transfer great amounts of pollutants to aerial parts.

Ttran et al. [37] investigated biomass obtained from phytoremediation energy crops M. x giganteus and Sida hermaphroditat planted on soil contaminated by heavy metals. Based on the results, they state that M. x giganteus demonstrated the ability for higher heavy metals uptake than Sida hermaphroditat. These results document that Miscanthus tolerates slightly polluted soil environments by heavy metals (Pb, Cd, Zn).

Similar findings are reported by Zadel et al. [38], i.e., M. x giganteus is a high-biomass-producing plant that tolerates heavy metals. Therefore, Miscanthus is of interest for nature-based solutions implementation in areas contaminated by heavy metals (use in phytoremediation) and for energy production.

Also, Nsanganwimana et al. [3] noted the tolerance of M. x giganteus to abiotic stress (e.g., also to pollutants) and recommended using the potential of this species for phytomanagement.

They state that the non-invasive M. x giganteus is more adapted for the implementation of nature-based solutions in soil remediation than its parent species (M. sinensis).

Nurzhanova et al. [39] studied the benefits of M. x giganteus cultivation at soils obtained from mining and ex-military sites polluted by As, Pb, Zn, Co, Ni, Cr, Cu, V, Mn, Sr, and U and also in the soil which has undergone anthropogenic contamination by Zn and Pb. The results revealed that M. x giganteus was able to resist the effects of heavy metals (tolerance index>1) and also that the most significant proportion of heavy metals gathered in the root system.

Andrejic et al. [40] assessed photosynthetic performance in M. x giganteus hybrid grown at high Zn concentrations. Despite the excess of Zn in the leaves, there was no serious reduction in the maximum quantum yield of PSII photochemistry, pointing to a high photosynthetic capacity and elevated tolerance to high Zn concentrations, and the ability of M. x giganteus to grow on Zn-polluted soils.

Kayama [31] reported that M. sinensis appears to be highly tolerant to Al when compared with other plant species. It has been observed to grow under acidic conditions with high Al concentrations [31]. M. sinensis, however, appears not to accumulate large amounts of Al, but rather it excretes high amounts of citric acid to form Al chelates that protect roots from Al toxicity [31]. M. sinensis is tolerant not only to Al but also to 0.015 mM chromium (Cr) and 0.2 mM zinc (Zn) as compared to other species (e.g., Andropogon virginicus and Sparganium eichhornii) [41]. M. x giganteus is tolerant to Cr [42] and cadmium [43] at certain concentration levels, but not enough to allow optimal crop growth rates.

Pavel et al. [44] investigated the addition of red mud in the cultivation of Miscanthus sinensis x giganteus in the contaminated soil by-product of the alumina industry, as a soil amendment on highly contaminated soils in the vicinity of a former Pb-Zn smelter in Coppenrath, Japan.
a Mică (Romania). They did not evaluate the reaction of the photosynthetic apparatus (which was the subject of our research), but the mobility of heavy metals (Zn, Cd, and Pb) in the soil, and their uptake and effects on growth and productivity of Miscanthus sinensis x giganteus. Overall the results suggest that Miscanthus sinensis x giganteus is a valuable energy plant and can be successfully grown on heavily contaminated soils with Zn, Cd and Pb. Moreover, the addition of red mud to these soils can lead to a significant decrease of heavy metals concentration in the soil and metal uptake by plant tissues.

The highest sensitivity to stressors displayed by M. x giganteus was in changes in the content of chlorophyll. This effect may be caused not only by the investigated abiotic stressors but also by the high variability of this trait (min 8.900 CCI, max 28.600 CCI, coefficient of variation 23.36 %).

The lowest chlorophyll content (10.900 and 14.833 CCI) was recorded with treatments C3 and B3, respectively. On the contrary, several times, treatments A1 and B1 showed higher values (25.433 and 26.500 CCI). The differences between these variants were significant.

In the control treatment, chlorophyll content was 19.267 CCI. Several treatments with an environmental stressor (A1 and B1) had a significantly higher level of chlorophyll than the content of the control treatment. Variants A1 (25.433 CCI) and B1 (26.500 CCI) had significantly higher chlorophyll concentrations than the control, but at graded doses of both salinity and acidity, we observed a significant reduction in chlorophyll content. For variant A1 from 25.433 CCI to 18.700 CCI (A3), variant B1 from 26.500 CCI to 14.833 CCI (B3). A similar trend of M. x giganteus chlorophyll content reduction with increasing salinity was observed by Stavridou et al. [28]. They stated that plants treated with higher salt concentrations (NaCl) showed a significant reduction of chlorophyll content compared to plants treated with lower salt doses and also to control plants.

The chlorophyll concentration was also affected by the number of days of stressor application.

In the first term of the measurement, we recorded the highest level of chlorophyll (19.685 CCI), significantly decreasing in the 2nd resp. the 3rd measurement dates (18.031 respectively 17.954 CCI). The plants of the control variant had a balanced chlorophyll concentration throughout the experiment. A similar trend in the chlorophyll content of M. x giganteus is reported by Stavridou et al. [27]. The relative chlorophyll content remained constant during the experiment in control plants and changed significantly over time. At higher salt concentrations, the chlorophyll level decreased during the experiment.

The results of the inorganic risk elements impact from the industry (solid pollutants and red mud) on the chlorophyll content of M. x giganteus are not known in the scientific literature.

In our assessment, especially for variants C3 and D3, we recorded a statistically significant reduction of chlorophyll content (10.900 respectively 16.100 CCI) compared to the control (19.267 CCI). To illustrate, we present at least the results published by Nurzhanova et al. [39]. They observed a significant decrease in the chlorophylls’ total content (chlorophylls a, b) in the leaves of plants when growing the said hybrid on soil artificially contaminated by Zn and Pb. The above information essentially corresponds to our results, which we obtained when assessing the impacts of solid pollutants and red mud on chlorophyll content.

Conclusions

The impact of different doses of abiotic chemical stressors (salinisation; acidification; inorganic risk elements from the industry), as well as the dates of three measurements of changes in the values of quantum yield PSII and chlorophyll content of M. x giganteus hybrid, were recorded.

The values of effective quantum yield PSII Y (II) varied from 0.561 (stressor acidity pH 6) to 0.693 (stressor acidity pH 5). No significant differences within the treatments were found, and we can conclude that the evaluated abiotic stressors did not affect the variability of this trait.

By contrast, maximum quantum yield PSII Fv/Fm showed a more sensitive response to abiotic stressors. During the growing season, the lowest value (0.689) was recorded with the solid pollutant stressor MgO (dose 3 kg). This dose induced the strongest stress response in the plants.

In contrast, the hybrid tolerated best the application of acidity stressors pH 6 (Fv/Fm = 0.767) and red mud, dose 1 kg (Fv / Fm = 0.775). The other evaluated doses of stressors were tolerated by the plants without tissue damage. Similarly to effective quantum yield PSII Y, no significant differences were recorded for dates of measurement. M. x giganteus displayed relatively good tolerance to the abiotic environmental load, and its quantum yields PSII did not show any significant destruction.

The highest sensitivity to stressors displayed by M. x giganteus was in changes in the content of chlorophyll. This effect may be caused not only by the investigated abiotic stressors but also by the high variability of this trait (min. 8.900 CCI, max. 28.600 CCI, coefficient of variation 23.36%).

The lowest chlorophyll content (10.900 and 14.833 CCI) was recorded with the solid pollutant stressor MgO (dose 3 kg) and acidity pH 4 - respectively. On the contrary, several times, treatments with saline (dose 20 g NaCl/2 dm³ H₂O) and acidic (pH 6) stressors showed higher values (25.433 and 26.500 CCI respectively). The differences between these variants were significant. The saline (dose 20 g NaCl/2 dm³ H₂O) and acidic (pH 6) stressors had...
significantly higher chlorophyll concentrations than the control (19.267 CCI); but, at graded doses of both salinity and acidity, we observed a significant reduction in chlorophyll content.

The key factors in the promotion of nature-based solutions within the context of sustainable management of Miscanthus sp. on contaminated land, based on obtained results, are that Miscanthus x giganteus is well tolerant of selected abiotic chemical stressors because the response of plants to induced stress was indicated a high level of resistance, with no damage to the photosynthetic apparatus having been observed. The evaluated hybrid is suitable for sustainable cultivation on contaminated soil, as the hybrid is tolerant (as evidenced by the results obtained by the reaction of the photosynthetic apparatus - fluorescence parameters and chlorophyll content) to the evaluated stressors and, reduces the transfer of contaminants into the food chain; this phytomanagement, based on nature-based solution, can mitigate both the environmental and health risks posed by pollutants and restore ecosystem services. Miscanthus x giganteus has very good preconditions for cultivation as an energy crop, be it within a mildly contaminated soil environment, devastated areas or areas intended for reclamation.

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Conflicts of Interest

The authors declare no conflicts of interest.

References

6. FIGALA J., VRANOVA V., REJSEK K., FORMANEK P. Giant miscanthus (Miscanthus x giganteus Greef Et Deu.) – A Promising Plant for Soil Remediation. Acta Universitatis Agriculturae et Silviculuriae Mendelianae Brunensis, 63 (6), 2241, 2015.
8. KORZENIOWSKA J., STANISLAWSKA-GLUBIAK E. Phytoremediation potential of Miscanthus x giganteus and Spartina pectinata in soil contaminated with heavy metals. Environmental Science and Pollution Research, 22 (15), 11648, 2015.


26. PLAZEK A., DUBERT F., KOŚCIELNIAK J., TATRZANSKA M., MACEJEWSKI M., GONDEK K., ZUREK G. Tolerance of *Miscanthus x giganteus* to salinity depends on initial weight of rhizomes as well as high accumulation of potassium and proline in leaves. Agricultural Crops and Products, 52, 278, 2014.


30. LEE D.K., PARRISH A.S., VOIGT T.B. Switchgrass and *Andropogon virginicus* Anders. are tolerant to multiple metal stresses including aluminum, heavy metals and oxidative stresses. Plant Cell Reports, 27, 951, 2008.


