

PHOTOSYNTHETIC APPARATUS EFFICIENCY OF *SIDA HERMAPHRODITA* CULTIVATED ON HEAVY METALS CONTAMINATED ARABLE LAND UNDER VARIOUS FERTILIZATION REGIMES

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Abstract

Contaminated and marginal lands are favourable place for biomass feedstock establishment, especially due to European Union directive 2009/28/EC. This strategy not only cover local demand for energy and heat but also can be valuable in those land phytomanagment. The second-generation perennial energy crop species are the most feasible for such purpose. We studied the impact of two different fertilizer treatments on plant physiological parameters associated with photosynthesis, heavy metals (HMs) and primary macronutrients accumulation in *Sida hermaphrodita* cultivated on HMs contaminated soil under field conditions. NPK fertilized plants showed the highest values of photosynthetic parameters at the beginning of growing season when compared to control and microbial inoculated plants. However, at the end of the growing season inoculated and control plants showed better photosynthetic performance than NPK treated. NPK fertilizer caused higher Cd and Zn shoot concentrations while microbial inoculation caused higher K and the lowest N and P concentrations in shoot. Due to Cd, Pb and Zn concentrations in plants which should not result in alleviation of photosynthetic apparatus efficiency and biomass production it could be summarize that *Sida hermaphrodita* is a suitable plant for cultivation on land contaminated with HMs under different fertilization regimes.

Keywords: photosynthesis rate, chlorophyll fluorescence, Cd, Pb, Zn, senescence

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1. INTRODUCTION

Biomass is an organic material which derived from plants (including algae, trees and crops), strictly from conversion CO₂, water, mineral nutrients and the sunlight in to carbohydrates, which are plant structure building matter, and oxygen via photosynthesis [1]. The process and strength of photosynthesis directly affect the yield and quality of plants [2]. Besides the classic measurements of photosynthesis activity of plants by gas-exchange analysis, chlorophyll fluorescence allow evaluating photosynthesis apparatus status of plants based on detection changes in some photosystem II (PSII) components, electron transport chain components, and light dependent photochemical reactions [3,4]. Additionally, chlorophyll content has great importance in estimation photosynthetic apparatus efficiency and general plant health [5].

Due to conventional energy sources decrement any environmental-saving technology, especially in industry, are desirable. Limitation of conventional heat production technology, based mainly on fossil fuels for increase usage of bio-based energy sources, especially biomass is the first step to improve environment condition.

Renewable energy directive (RED) 2009/28/EC [6] promote usage of renewable energy sources in the members of European Union. These countries have to increase the share of renewable energy of gross final energy consumption to 20 % in 2020. That directive also excludes several land categories from biomass production, especially lands with high biodiversity value and high carbon stock, what made marginal and degraded lands a favourable place to energy crops cultivation [7].

Marginal lands could be contaminated by many different compounds or/and harmful elements, which have got anthropogenic or environmental origin. That contamination can significantly affect the quality of agricultural products [8]. Heavy metals could alter the photosynthetic activity indirectly, decreasing the content of photosynthetic pigments or damaging the photosynthetic apparatus on every level of its organization [9]. For this reason, analysis of energy crops photosynthetic apparatus efficiency, while growing on contaminated soil is very important.

Cultivation of energy crops on such contaminated soil especially on heavy metal contaminated (HMC) soil can be profitable not only in biomass production but also in phytoremediation [10].

Nowadays biomass production is focused on second generation bioenergy crops. Such plants have got less inputs requirement, produce more energy and reduce greenhouse gases emission more than annual crop species belonging to first generation [11]. *Sida hermaphrodita* as a C₃ photosynthesis perennial plant species was already cultivated for energy purposes [12,13].

The aim of the study was to assess influence of two different fertiliser treatments on plant physiological parameters associated with photosynthesis, heavy metals (HMs) and primary macronutrients accumulation in *S. hermaphrodita* cultivated on HMs contaminated soil under field conditions.

2. MATERIALS AND METHODS

2.1. Site description

The experimental plots were established on contaminated arable land in Bytom (Upper Silesia), Poland (50°20'43.1"N 18°57'17.9"E) at the beginning of the 2014 growing season, on the area lease by the Institute for Ecology of Industrial Areas. Soil contamination with zinc, cadmium and lead on described area resulted from Pb/Zn smelting activity over the last century. Total soil heavy metals (HMs) concentration exceed the threshold values proscribed by Polish government regulation [14], excluding this area from food production. Yearly average values of temperature and sum of precipitation measured during the third growing season (May 2016 – September 2016) were 17.5°C and 450 mm respectively (Institute of Meteorology and Water Management, Poland).

2.2. Experiment design

Roots seedlings of *Sida hermaphrodita* were planted at 10 cm depths from direct root cuttings which were originated from an uncontaminated site and pre-cultivated in controlled conditions. In the middle of the May 2014 on each from three plots 49 plants were planted over an area of 16m² (3 plants per 1m²) with a buffer zone of 4m between each plot which protected plants against uncontrolled fertilisation. Single plot trials with pseudo-replication were performed due to high soil homogeneity on the field before experiment establishment. During plantation establishment, each plot was treated in different way:

- SH I – control, untreated plot
- SH II – plot treated with standard NPK chemical fertilizers (ammonium sulphate and Polifoska - 4% N, 22% P₂O₅, 32% K₂O) once before planting. Dose was adjusted according to El Bassam [15] and amounts of applied macronutrients were as follow: nitrogen 100 kg ha⁻¹, phosphorus 80 kg ha⁻¹ as P₂O₅ and potassium 120 kg ha⁻¹ as K₂O.
- SH III – plot treated with microbial inoculum (Emfarma Plus® ProBiotics, Poland) which consist of Lactic Acid Bacteria > 3.0 × 10⁵ CFU ml⁻¹, Yeast < 1.0 × 10⁶ CFU ml⁻¹ and Purple Non-Sulfur Bacteria > 1.0 × 10⁴ CFU ml⁻¹ in molasses suspension. Inoculum (10% water solution) was applied on roots and on soil surface (8l per plot) before planting. In addition, plant leaves were treated monthly during the growing season by aerosol treatment (8l per plot).

2.3. Soil physico-chemical parameters

Results from initial soil physico-chemical analyses including: pH, EC, organic matter content (OM), concentration of elements N, P, K, Mg, Ca, Fe, Pb, Cd, Zn as well as bioavailable forms of analysed heavy metals (Pb, Cd, Zn) were previously reported for investigated plots by Pogrzeba et al. [12] (Table 1).

Table 1. Soil physico-chemical characteristic according to Pogrzeba et al. [12]

Soil parameters	Experimental variants		
	SH I	SH II	SH III
pH	6.50 ± 0.04a	6.48 ± 0.06a	6.58 ± 0.09a
EC (µS cm ⁻³)	87.57 ± 3.25a	87.72 ± 1.67a	89.44 ± 2.29a
OM (%)	6.20 ± 0.16a	6.22 ± 0.23a	6.52 ± 0.27a
N (%)	0.18 ± 0.01a	0.17 ± 0.01a	0.18 ± 0.01a
P (g kg ⁻¹)	1.02 ± 0.01a	0.99 ± 0.00a	0.99 ± 0.00a
K (g kg ⁻¹)	0.98 ± 0.00a	0.97 ± 0.00a	1.02 ± 0.01a
Mg (g kg ⁻¹)	2.22 ± 0.01b	2.33 ± 0.01b	2.94 ± 0.01a
Ca (g kg ⁻¹)	4.50 ± 0.02b	4.83 ± 0.01b	5.60 ± 0.03a
Fe (g kg ⁻¹)	11.85 ± 0.01a	11.96 ± 0.02a	12.10 ± 0.04a
Pb (mg kg ⁻¹)	635.6 ± 3.7a	637.7 ± 5.4a	639.1 ± 8.1a
Cd (mg kg ⁻¹)	25.70 ± 0.48a	26.01 ± 0.28a	26.04 ± 0.38a
Zn (g kg ⁻¹)	2.36 ± 0.01a	2.42 ± 0.01a	2.46 ± 0.01a
Pb _{bio} (mg kg ⁻¹)	LOQ	LOQ	LOQ
Cd _{bio} (mg kg ⁻¹)	1.87 ± 0.1a	1.85 ± 0.07a	1.71 ± 0.18a
Zn _{bio} (mg kg ⁻¹)	110.10 ± 6.67a	107.42 ± 4.11a	95.66 ± 13.19a

SH I – control plots, SH II – NPK fertilized plots, SH III – microbial inoculated plots, EC – electrical conductivity, OM – organic matter,

bio – bioavailable fraction of heavy metals, LOQ – limit of quantification.

A lower-case letters (a, b) denotes significant differences among soils samples taken from different plots at $P \leq 0.05$ according to Fisher LSD test.

Values are means ± SE (n = 3)

2.4. Gas exchange, plant pigment content and chlorophyll *a* fluorescence measurements

Gas exchange measurements were performed monthly (June – August) on each plot using Infra-Red Gas Analyser (Lc ProSD, ADC Bioscientific, UK) with measuring chamber set on climate condition (21°C, 1500 PAR, CO₂ ambient) For

measurements from each plot 3 plant were selected and on each plant measurements were performed on two young fully developed leaves.

Plant pigment content measurements were performed monthly (June – August) on each plot using plant pigment content meter (Dualex Scientific+, FORCE-A, France). Measurements were performed on the same plants where gas exchange measurements were performed.

Chlorophyll *a* fluorescence measurements were performed monthly (June – August) on each plot using fluorimeter (Handy Plant Efficiency Analyzer, Hansatech Instruments Ltd, UK). Measurements were performed at night when samples were adapted to darkness, on the same plants where gas exchange and plant pigment content measurements were performed.

2.5. Concentration of heavy metals and primary mineral macronutrients in plants

Three plant shoots from each of investigated plants were collected at the end of third growing season (October 2016) on each plot. Concentration of metals in the plant shoots was determined by hot plate digestion and flame atomic absorption spectrometry (SpektrAA 300, Varian INC., USA). Plant samples were digested in nitric and perchloric acid (4:1 v/v) [16]. Total nitrogen concentration (N) in plant shoots was assessed using the method described by Bremner [17], whereas total phosphorus (P) and potassium (K) concentration in plant shoots were assessed using ICP (Liberty 220, Varian, USA) in previously digested samples.

2.6. Statistical analysis

One-way ANOVA followed by post-hoc LSD test at $P \leq 0.05$ were used to distinguish differences between experimental variants during each month as well as to distinguish differences between values obtained during June, July and August measurements separately for each experimental variant.

3. RESULTS AND DISCUSSION

3.1. Soil characteristics

Physical and chemical soil parameters are presented in Tab. 1 according to Pogrzeba et al. [12]. Heavy metal concentrations in the soil exceeded Polish limits for food crop production [18]. The pH was almost neutral, followed by high content of organic matter (OM) and low electrical conductivity (EC). Despite low Pb bioavailability, presented results indicate high concentration of Cd and Zn bioavailable forms. There is almost no differences between plots in initial soil characteristic what indicate that soil is highly homogenous.

3.2. Gas exchange parameters

Gas exchange parameters are presented in Fig. 1. Photosynthesis rate (Fig. 1a) showed no differences between experimental variants within each month, while considering differences between month, it was found that photosynthesis rate decrease consequently from June to August. Despite the fact that there were no differences between experimental variants, there is tendency suggesting that NPK treated plants showed the best photosynthesis rate in the June while the worst in the August.

The highest transpiration rate (Fig. 1b) were found in June for plant treated with chemical fertilizer, lower for inoculum treated plants and the lowest for control. It was found that there were no differences between experimental variants in August, however obtained values were about 43% lower when compare to June measurements. Values obtained during August measurements indicate the same tendency between experimental variants as found in photosynthesis rate. Transpiration rate measured in June was the highest among all experimental variants. Values obtained in August were about 30% higher when compare to those obtained in July.

There is dearth of papers considering direct measurement of photosynthesis using IRGA on *Sida hermaphrodita*. Kocoń and Jurga [13] performed this type of measurements on *S. hermaphrodita*, however those were performed once a year, in spring, on young plants, with use of different chamber set up than used in the present study. Rosenthal et al. [19] performed continuous measurements of photosynthesis on *Glycine max* during two growing seasons (from June – September). They reported start of photosynthesis rate parameter decrease at the turn of August and September. Constant decrease of photosynthesis rate during growing season in present report could be associated with presence of HMs in soil and induced senescence by reactive oxygen species (ROS) [20], this fact could be additionally supported by month by month decreasing tendency visible for values obtained for chlorophyll content and chlorophyll fluorescence vitality index (F_v/F_m).

3.3. Plant pigment content

Chlorophyll content measured in June showed the highest value for plants treated with chemical fertilizer and untreated, significantly lower values were found for inoculated plants. In July, it was found increasing tendency of chlorophyll content for control and inoculum treated plants while decrease was reported for NPK treated. The chlorophyll content in July was higher by about 3 relative units (r.u.) in control and inoculum treated plants when compare to NPK fertilized. In August decrease of chlorophyll content in all experimental variants was found. There were no statistically significant differences between experimental variants, however tendency with the lowest value for NPK treated plants still occur in August.

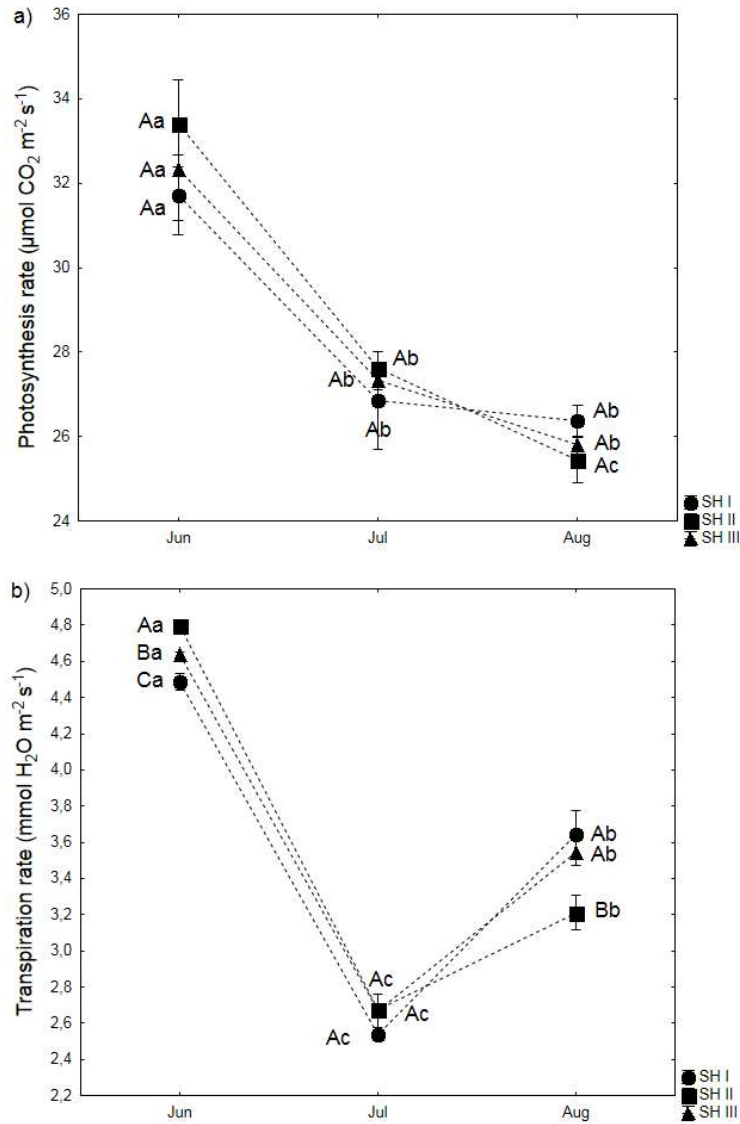


Fig. 1. Influence of NPK fertilization (SH II) and microbial inoculation (SH III) on (a) photosynthesis rate and (b) transpiration rate of *Sida hermaphrodita* in June, July and August 2016 growing season. Values are means \pm SE ($n = 18$). Capital letters (A, B, C) denote significant differences among parameters in plants on different plots within one month, while lower case letters (a, b, c) denote significant differences among parameters between months within one experimental variant at $P \leq 0.05$ according to Fisher LSD test.

Anthocyanins content was on the same level in each experimental variant on June and July. High decrease of anthocyanins was found in August for each experimental variant when compare to June and July. Although, there were no statistically significant differences between experimental variants the highest anthocyanins content was found for plant treated with NPK fertilizer while the lowest for plants treated with microbial inoculum.

The progression of leaf senescence is most commonly monitored as chlorophyll degradation [21, 22]. Anthocyanin accumulation begins shortly after the onset of chlorophyll decline, typically before any visible change in leaf color appear. This could demonstrate a direct association between anthocyanin production and the period of increased vulnerability to photoinhibition during senescence, and suggest that anthocyanins may perform a photoprotective role in autumnal foliage. In addition, under stressful conditions, even moderate irradiances can induce photoinhibitory damage to plants [23]. This statement could additionally be supported by the fact that there were significant negative Pearson's correlations between anthocyanins and chlorophyll ($r = -0.621$, $p=0.000$), photosynthesis rate ($r = -0.399$, $p = 0.000$) and F_v/F_m ($r = -0.435$, $p = 0.000$)

3.4. Chlorophyll *a* fluorescence parameters

The most common parameters obtained from chlorophyll *a* fluorescence measurements are F_v/F_m (Maximum quantum yield of primary photochemical reactions) that indicates the probability of trapping the energy of absorbed photons by PSII reaction centres and PI_{ABS} (Performance index) an indicator of PSII functional activity normalized to the absorbed energy [24,25].

Those parameters are usually described as vitality indices [25]. The F_v/F_m parameter was the highest for fertilized plants while the lowest for control plants in June. There were no differences between experimental variants in July, however in August NPK fertilized plants showed the lowest F_v/F_m values when compare to the other experimental variants. General tendency indicate that F_v/F_m parameter consequently decrease from June to August. Performance index showed slightly different behavior when compare to F_v/F_m . The PI_{ABS} in June was 23% lower in control plants and those treated with microbial inoculum when compare to NPK fertilized plants. There were no differences between experimental variants in July while considering PI_{ABS} parameter. The control plants showed significantly lower values of PI_{ABS} when compare to inoculum treated plant in August. There were no differences between NPK treated plants and other experimental variants. Control plants did not differ significantly between months. Inoculum treated plants had the same value of PI_{ABS} in June and July while decrease of this parameter was reported in August. The PI_{ABS} measured for NPK treated plants decreased in July when compare to June, however it increased in August when compare to July to value similar to this obtained in June.

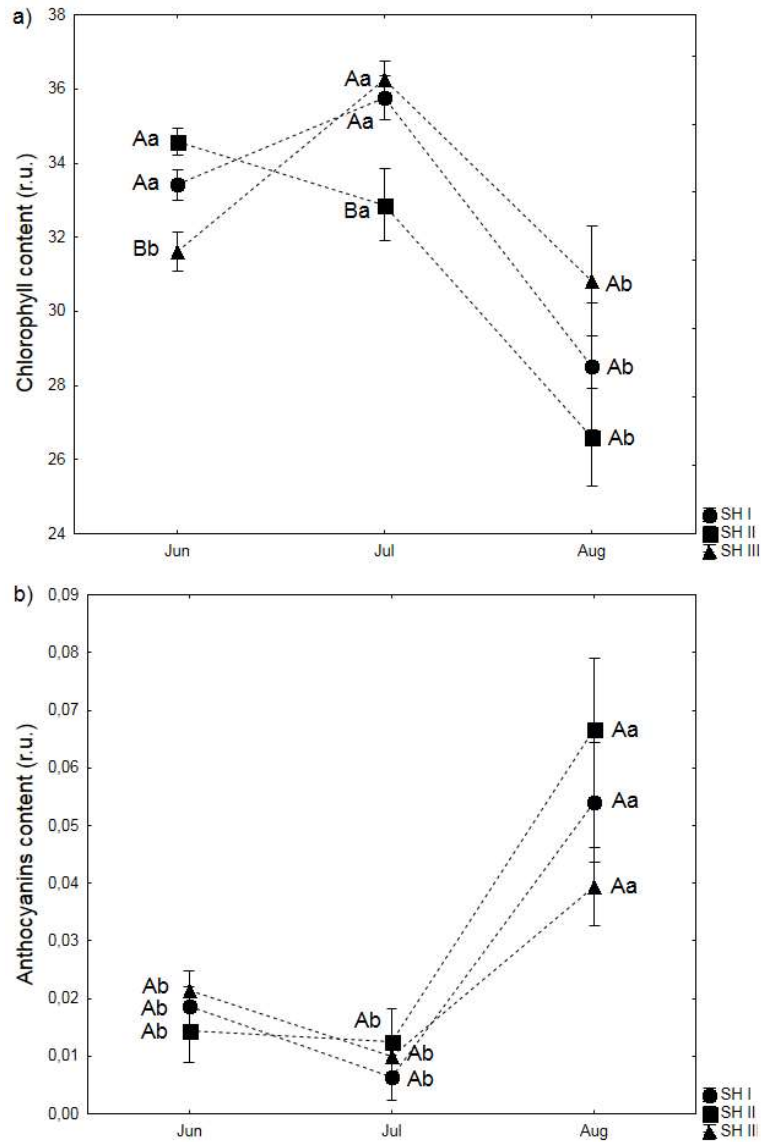


Fig. 2. Influence of NPK fertilization (SH II) and microbial inoculation (SH III) on (a) chlorophyll content and (b) anthocyanins content of *Sida hermaphrodita* in June, July and August 2016 growing season. Values are means \pm SE ($n = 12$). Capital letters (A, B, C) denote significant differences among parameters in plants on different plots within one month, while lower case letters (a, b, c) denote significant differences among parameters between months within one experimental variant at $P \leq 0.05$ according to Fisher LSD test.

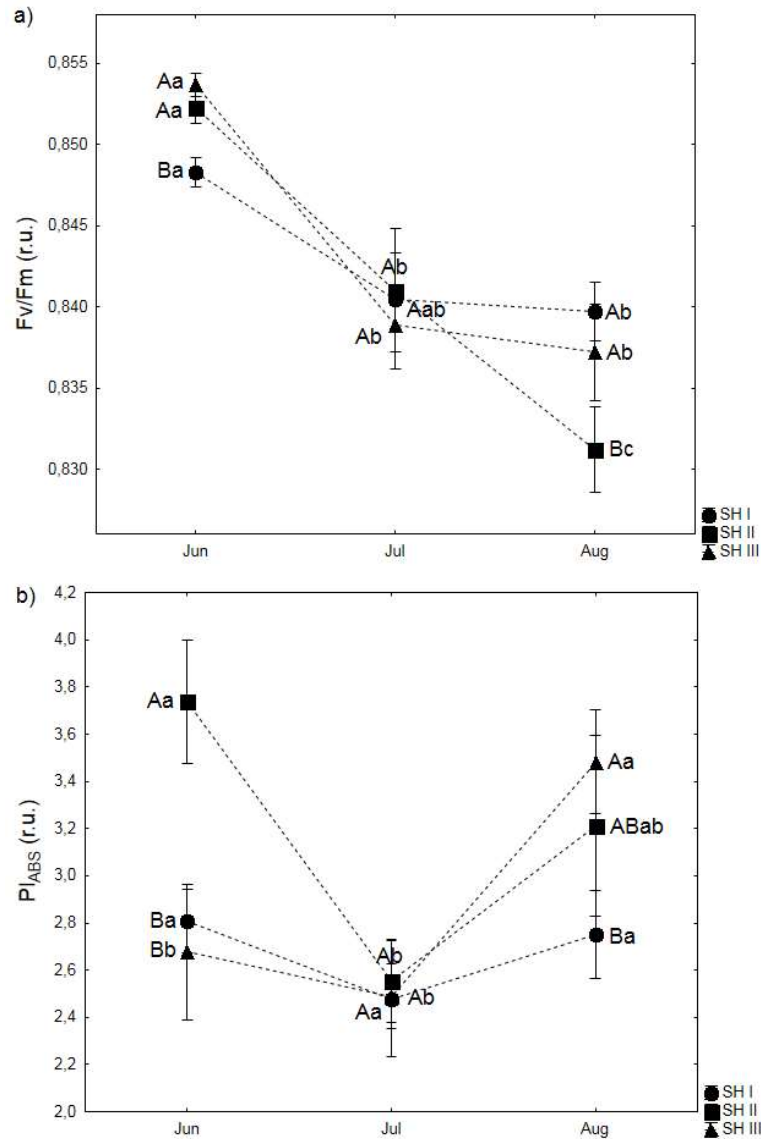


Fig. 3. Influence of NPK fertilization (SH II) and microbial inoculation (SH III) on (a) F_v/F_m and (b) PI_{ABS} of *Sida hermaphrodita* in June, July and August 2016 growing season. Values are means \pm SE (n = 12). Capital letters (A, B, C) denote significant differences among parameters in plants on different plots within one month, while lower case letters (a, b, c) denote significant differences among parameters between months within one experimental variant at $P \leq 0.05$ according to Fisher LSD test.

3.5. Heavy metals and mineral macronutrients concentration in plant shoots

Heavy metals and mineral macronutrients concentration in plants shoots was presented on Table 2. It was found that on each plot Pb concentration was below detection limit, this phenomenon could be associated with low bioavailability of this element in the soil [26]. Cadmium concentration in shoots was the highest in NPK fertilized plants, while it was about 24% lower in control and inoculum treated plants. Similar tendency was found for Zn shoot concentration, however control and NPK fertilized plants showed significantly higher values by about 30% and 41%, respectively when compare to inoculum treated plants. Control plants showed the highest shoot N concentration among experimental variants. It was 0.23% and 0.30 % higher when compare to NPK and inoculum treated plants respectively. Similar tendency was observed for shoot P concentration. The highest shoot K concentration was found simultaneously for NPK and inoculum treated plants and it was nearly 20% higher when compare to control plants.

The highest heavy metals concentration in NPK treated plant shoots (particularly Cd and Zn) could be associated with application of chemical fertilizer which could increase the mobility of heavy metals by reducing pH value. This phenomenon occurs due to the presence of NH_4^+ in chemical fertilizers as the N-source, which results in H^+ extrusion by the roots and acidification of the soil [27]. Similar phenomenon was observed for *Miscanthus x giganteus* on the same field [28]. Although, concentration of Cd and Zn in plants shoots did not exceed toxic concentration describe by Kabata-Pedias [29] it could slightly affect photosynthetic apparatus efficiency, especially in senescent plants due to its higher sensitivity to stress factors. This could explain results obtained for NPK fertilized plants comparing to other treatments. The highest value of chlorophyll content, F_v/F_m , photosynthesis rate and transpiration rate in June for NPK fertilized plants could be associated with better supplementation, however the lowest value of those parameters for the same plants in August could be associated with combined effect of senescence and the highest HM concentration in NPK fertilized plants shoots. The lowest concentration of N and P in inoculated plant shoots could be associated with competition between plant roots and applied monthly microorganisms as well as indigenous rhizosphere microorganisms. This phenomenon could be associated with molasses included in inoculum formula, which derived easily decomposable C and prime microorganisms (microbial activation) for achieving additional N from soil organic matter decomposition [30].

Table 2. Heavy metals and primary macronutrients concentration in plant shoots

	SH I	SH II	SH III
Pb (mg kg ⁻¹)	LOQ	LOQ	LOQ
Cd (mg kg ⁻¹)	2.46 ± 0.47b	3.06 ± 0.10a	2.23 ± 0.39b
Zn (mg kg ⁻¹)	163.3 ± 8.6b	195.7 ± 12.8a	117.2 ± 10.1c
N (%)	0.74 ± 0.03a	0.51 ± 0.03b	0.44 ± 0.03c
P (mg kg ⁻¹)	866.2 ± 66.9a	659.6 ± 72.6b	511.2 ± 42.6c
K (g kg ⁻¹)	9.28 ± 0.87b	11.11 ± 0.56a	11.66 ± 1.11a

SH I – control plots, SH II – NPK fertilized plots, SH III – microbial inoculated plots, LOQ – limit of quantification. A lower-case letters (a, b, c) denotes significant differences among elements concentration in plant shoots taken from different plots at $P \leq 0.05$ according to Fisher LSD test. Values are means ± SE (n = 9)

4. CONCLUSIONS

In conclusion, application of different fertilizers did not affect photosynthesis apparatus efficiency when compared to control. Heavy metals concentration in shoots did not exceed toxic concentration thresholds for higher plants. Based on chlorophyll and anthocyanins content it could be assumed that plant senescence begins at turn of July and August. Senescence could increase vulnerability of photosynthetic apparatus to heavy metal toxicity. It could be summarized that *Sida hermaphrodita* is plant suitable for cultivation on land contaminated with HMs under different fertilization regimes.

5. ADDITIONAL INFORMATION

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WYDAJNOŚĆ APARATU FOTOSYNTETYCZNEGO *SIDA HERMAPHRODITA*
UPRAWIANEGO NA GLEBIE ZANIECZYSZCZONEJ METALAMI CIĘŻKIMI
W WARUNKACH ZRÓŻNICOWANEGO NAWOŻENIA

Streszczenie

Tereny zanieczyszczone i odłogowane stanowią opcję dla uprawy roślin na cele energetyczne, szczególnie jeśli weźmie się pod uwagę rozporządzenia dyrektywy Unii Europejskiej w sprawie promowania stosowania energii ze źródeł odnawialnych (2009/28/EC). Spełnienie jej wymogów może być po części zrealizowane poprzez uprawę roślin energetycznych drugiej generacji. Zastosowanie takiego podejścia pozwoli nie tylko pokryć lokalne zapotrzebowanie na odnawialne źródła energii, ale także wspomóc zarządzanie terenami nieprzydatnymi w produkcji roślin na cele żywnościowe oraz paszowe. W przedstawionej pracy badano wpływ zastosowania zróżnicowanego nawożenia na parametry fizjologiczne ślazuca pensylwańskiego uprawianego na glebie zanieczyszczonej metalami ciężkimi. Rośliny nawożone standardowymi nawozami mineralnymi charakteryzowały się wyższymi wartościami parametrów fizjologicznych na początku sezonu wegetacyjnego, podczas gdy na końcu sezonu wegetacyjnego wyższe wartości stwierdzono dla roślin w wariantach kontrolnym i szczepionką mikrobiologiczną. Nawożenie mineralne spowodowało wyższe zawartości Cd i Zn w roślinach, podczas gdy rośliny traktowane szczepionką mikrobiologiczną miały wyższe zawartości K oraz najniższe zawartości N i P w częściach nadziemnych. Stwierdzone zawartości Pb, Cd i Zn w częściach nadziemnych ślazuca pensylwańskiego nie powinny mieć negatywnego wpływu na stan aparatu fotosyntetycznego i wydajność produkcji biomasy. W związku

z tym rośliną ta może być przydatna w produkcji biomasy na cele energetyczne na terenach zanieczyszczonych.

Słowa kluczowe: Natężenie fotosyntezy, fluorescencja chlorofilu, Cd, Pb, Zn, starzenie

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