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Solid biofuels properites of *Miscanthus X giganteus* cultivated on contaminated soil after phytoremediation process

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ABSTRACT

Following the objectives of the 2030 climate and energy framework as well as the European Green Deal strategy to 2050, renewable energy sources have been defined as an important factor in the process of climate change mitigation. Renewable energy sources are the basis of the aspirations of the EU to achieve low-carbon energy self-sufficiency. Under the classification of renewable energy sources biomass obtained by cultivating energy crops is also included. In order to avoid collisions between energy production and food/feed production, the cultivation of energy crops should be based on marginal soils, which also include soil contaminated by heavy metals. The use of energy crops in the process of phytoremediation is a multifunctional methodology that manifests itself through the cleaning of contaminated soil with the biomass production. The usefulness of using certain biomass types in the ignition process is valorized by the determination of biomass properties. The aim of this three years' investigation was to analyze the biomass quality (proximate and ultimate analysis, calorific values, micro and macroelements) of *Miscanthus x giganteus* cultivated on soils with three cadmium (Cd) and mercury (Hg) contamination levels. All analyzed parameters, except Cd, K and Cr, are compatible with or have only expected differences from the literature data and/or ISO 17225–1:2014 norm for solid biofuels. The values of ash, volatile matter, C content and lower heating value as one of the most important energy parameters, are in the range 1.35–3.66%, 86.28–90.39%, 46.34–49.91% and 16.24–16.93 MJ/kg respectively.

1. Introduction

Biomass, as component of renewable sources, is the primary source of green energy in the EU with an average share of 63.5%. Concerning the total amount of energy produced from biomass, the largest share refers to the forest biomass with 70%, followed by the agricultural biomass with 18% and the organic waste with a share of 12% [1].

Generally, energy crops are defined as crops that produce significant amounts of biomass with minimal agro-technical investments. Considering their sub-origin, they can be divided as Short rotation coppice (e.g. Poplar, Willow, Salix) and Grassy energy crops (e.g. *Miscanthus x giganteus*, *Arundo donax*, *Panicum virgatum*). Such second-generation, non-food crops can be readily integrated into sustainable agricultural systems [2].

Depending on the individual species morphology as well as the method of crop management, biomass from energy crops can be utilized by all based and advance conversion technologies [3]. From ecological aspect, energy crops have relatively low global warming potential [4], high carbon sequestration potential (carbon storage), they enhance biodiversity and contribute to salinity and erosion mitigation [5,6].

In order to avoid negative aspects between biomass and food/feed production, the cultivation of energy crops should be focused on marginal soils [6,7] which also includes soils that are physically, biologically and/or chemical degraded [8]. Presence of heavy metals in the soil, that is considered as chemical degradation, may lead to series of negative impacts on soil and water quality [9]. Contaminated soils by heavy metals can be remediated by various methods and one of them is phytoremediation. Phytoremediation is considered as environmentally sustainable, energy efficient and low-cost biological technique, that is based on the plant possibility to absorb, immobilize or degradation of different pollutants from contaminated soils [10–12]. The usage of energy crops in the soil phytoremediation process is a beneficial option, especially in the context of linking bioenergy production and phytoremediation [13].

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Phytoremediation efficiency is usually limited by low toxicity tolerance, low above ground biomass and/or shallow root system [14–17]. *Miscanthus (Miscantus × giganteus Greef Et Deu.)* is defined as the second-generation energy crop [18] that has high above [19] and below ground biomass productivity [20], low input requirements (fertilizers, herbicides, pesticides) and relatively high stress tolerance [7, 21–23], and therefore high phytoremediation potential [24]. Although higher heavy metal concentrations in the soil have negative impact on *Miscanthus* plant productivity [25], phytoremediation has been proven as useful techniques for the treatment of Cd, Hg, Pb, As, Cr, Cu, Ni and Zn in contaminated soil [26–32]. The most common used remediation techniques for heavy metals are phytoextraction and phytostabilization are, that differ considering accumulation of pollutants in below and/or above ground biomass [33]. Nsanganwimana et al. [24] summarized that *Miscanthus* accumulate more trace elements in the below ground compared to above ground biomass.

Thermochemical conversion technologies (combustion, pyrolyses, torrefaction, gasification) are the conventional and promising pathways for utilization of biomass obtained from phytoremediation process [34]. Combustion of heavy metals contaminated biomass still results in regular emissions (CO, NOx, fly ash), and also produces solid and gaseous metal compounds [35]. Laval-Gilly et al. [36] determined that low metals concentrations in aboveground biomass should facilitate the contaminated biomass utilization, and Pogrzeba et al. [37] stated that biomass after phyremediation process should be treated as hazardous material.

In order to avoid the reemissions of metals into the atmosphere, especially those that are highly volatile at combustion temperature, combustion should be carried out in controlled conditions [35,37]. Proper and responsible disposal of ash obtained after the process of contaminated biomass combustion represents very important segment in ecologically sustainable way of energy production [38].

Solid biomass composition (determined by proximate and ultimate analysis, calorimetry for higher heating value and contents of micro and macro elements) is very important for the combustion process [39]. Biomass composition depends on agroecological conditions of cultivated sites i.e. soil type, climate factors, plantation age and plant management [40]. Most of the expected property values for various biomasses, including *Miscanthus* grass, can be found in ISO 17225–1:2014 *Solid biofuels – Fuel* specifications and classes norm.

Werle et al. [41] and Tran et al. [42] investigated solid biomass properties of *Miscanthus* cultivated on different polluted sites in Poland and Germany and determined deviations in proximate (eg. ash 4.20–6.50%, volatile matter 74.00–76.50%) and ultimate (eg. C 44.6–45.20%, N 1.13–2.15%) analyses that were related to different soil properties (pH value and heavy metal content) of cultivation sites. Considering further energetic utilization via thermochemical conversion, Uchman et al. [43] determines proximate (ash 1.36%, volatiles 75.4%; lower heating value 19.45 MJ/kg) and ultimate analyses (C 46.6%; H 7.16%; N 0.16%; S 1.35%; O 44.73%) of contaminated heavy metal *Miscanthus* biomass grown on metallurgical complex. On industrial polluted site, Laval-Gilly et al. [36] have determined significantly lower ash content in *Miscanthus* leaf grown on control site (4.60%) compared to contaminated site (13.60%).

The use of energy crops in the process of phytoremediation is a multifunctional methodology that manifests itself through the simultaneous cleaning of contaminated soil with the biomass production. Relatively few available papers deal with the topic of energy valorization of contaminated biomass, especially those that were grown on soil contaminated with mercury.

The current study represents the results and analysis of the effects of cultivating conditioning (three years; three Cd i Hg contamination levels) on the final quality of *Miscanthus x giganteus* biomass as solid biofuel. Furthermore, it was defined appropriate mathematical models for the contents of parameters grouped as proximate and ultimate analysis, calorimetry and contents of some micro and macro elements.

2. Material and methods

2.1. Materials

The experiment was set up in an open greenhouse in plastic experimental pots. Weather conditions (sunshine duration, temperature and precipitation) were natural and soil moisture was controlled daily and if necessary adjusted to field water capacity. The experiment was laid out according to the completely randomized design (Fig. 1.) and high-quality rhizomes was planted in four treatments in three replicates over three years.

Four treatments (C, L1, L1 + SS, L2) with different concentrations of Cd (0, 10 and 100 mg kg-1) and Hg (0, 2 and 20 mg kg-1) were applied to the soil (Table 1.). The first control group (C) consisted of pure soil while the soil of the second group was treated with a lower amount (L1) of pollutants: 10 mg Cd kg-1 (in CdO (s) form) and 2 mg Hg kg-1 (in HgCl2 (s) form). A third group (L1 + SS) was treated with identical concentrations of Cd and Hg applied to the soil as in L1, but with an addition of sewage sludge in an equivalent of maximum 1.66 t (dry matter – DM) ha-1 according to the Croatian legislation (OG NN 38/2008). The soil of the fourth group was treated with a higher content (L2) of pollutants: 100 mg Cd kg-1 and 20 mg Hg kg-1 of soil. The pollutants were applied as p. a. salts in solid phase (clean soil were mixed with an appropriate amount of salts to achieve homogeneity) to the dry soil before the first year of vegetation. The hydro meteorological conditions, soil and sewage sludge properties used in the experiment were explained in the detail in Zgorelec et al., [33].

2.2. Methods

2.2.1. Biomass sampling

The sampling of *Miscanthus,* for each experimental pot, was conducted in fallowing years 2015, 2016 and 2017 in early March. Samples were grounded in a laboratory mill (IKA Analysentechnik GmbH, Germany) after the natural air drying process. In order to provide repeatability of the analyses all parameters were analyzed at least three times.

2.2.2. Proximate analysis

The proximate analysis included determination of moisture content [44], by using the laboratory dryer (INKO, Croatia), ash [45] by using the muffle furnace (Nabertherm, USA) as well as fixed carbon and volatile matter content [46] which were calculated by computed derivation.

2.2.3. Ultimate analysis and heating value

The ultimate analysis included determination of carbon, hydrogen, nitrogen, and sulphur share which were determined by dry combustion method by using the Vario Macro CHNS analyzer (Elementar Analysensysteme GmbH, Germany), toward the protocols for carbon,

Fig. 1. The demonstration of the experimental setup.

Table 1
Different soil treatments varied in concentrations of Cd and Hg.

Treatment	Cd concentration	Hg concentration	Amendment
1 – Control	–	–	–
2 – Level 1	10 mg/kg soil	2 mg/kg soil	–
3 – Level 1 + SS	10 mg/kg soil	2 mg/kg soil	10 g sewage sludge*
4 – Level 2	100 mg/kg soil	20 mg/kg soil	–

*equivalent of maximal 1.66 t_{DM} ha^{-1} according to Croatian legislative.[51].

hydrogen, and nitrogen [47] and sulphur [48] determination while the oxygen share was calculated by difference.

The higher heating values (reported in MJ/kg on dry basis) were determined by the EN 14918:2010 method [49] using an adiabatic calorimeter (C200, IKA Analysentechnik GmbH, Heitersheim, Germany), while the lower heating value was calculated by computed derivation.

2.2.4. Minor, major and toxic elements

The quantity of major and minor elements was carried out by using atomic absorption spectroscopy (PerkinElmer, AAnalyst 400). Major elements (sodium, magnesium, potassium, and calcium) was determined by HRN EN ISO 16967:2015 method [50], while the content of the minor element (chromium) were determined by HRN EN ISO 16968:2015 method [51]. The samples for the analysis of major and minor elements quantity was previously prepared in the microwave oven. Total Cadmium and Mercury were determined using AAS, FIMS as described in Zgorelec et al. [33].

2.2.5. Quality control and statistical analysis

For all studied parameters quality control was conducted through accuracy and method precision by using reference materials (IPE 171 and IPE 186 for plant, Wageningen University) and by replication of some measurements in triplicates. Absolute error and relative standard deviation (RSD) of all measured parameters were in the prescribed limits by each norm.

For optimizing a variety of processes response surface methodology (RSM) was used [52–54]. The main reason of RSM use is reduced experimental runs number which provide adequate information for statistically valid results. The RSM equations describe the test variables effects on the remark responses, determine test variables interrelationships and show the all test variables combined effect in the remark responses.

The descriptive statistics of the collected data are presented using in Tables 2–4. The experimental data used for the analysis are derived using the two factors design (three years × four treatments), with 12 samples, Brlek, et al. [55]. The following second order polynomial (SOP) model was fitted to the exploratory data. Seventeen models of the accompanying structure are produced to relate 17 responses and two factors, for each of the breeding variables:

Table 3
Ultimate analysis for *Miscanthus x giganteus* samples (d.m.).

Treatment	Year	N (%)	C (%)	S (%)	H (%)	O (%)
1	1	0.406 ± 0.008^{a}	49.905 ± 0.349^{c}	0.316 ± 0.048^{a}	5.643 ± 0.395^{bc}	43.730 ± 0.105^{a}
1	2	0.411 ± 0.014^{a}	48.396 ± 0.453^{bc}	0.333 ± 0.075^{a}	5.370 ± 0.020^{abc}	45.490 ± 0.535^{ab}
1	3	0.412 ± 0.006^{a}	48.653 ± 0.493^{bc}	0.328 ± 0.065^{a}	4.990 ± 0.045^{abc}	45.617 ± 0.414^{ab}
2	1	0.407 ± 0.009^{a}	48.396 ± 0.603^{bc}	0.319 ± 0.011^{a}	5.680 ± 0.571^{c}	45.199 ± 0.706^{ab}
2	2	0.404 ± 0.011^{a}	46.343 ± 0.159^{a}	0.299 ± 0.014^{a}	5.242 ± 0.136^{abc}	47.713 ± 0.242^{c}
2	3	0.410 ± 0.005^{a}	49.122 ± 0.523^{bc}	0.291 ± 0.002^{a}	5.027 ± 0.092^{ab}	45.149 ± 0.601^{ab}
3	1	0.415 ± 0.014^{ab}	47.586 ± 0.695^{ab}	0.293 ± 0.019^{a}	5.409 ± 0.014^{abc}	46.297 ± 0.693^{bc}
3	2	0.439 ± 0.005^{b}	48.930 ± 0.514^{bc}	0.294 ± 0.011^{a}	5.330 ± 0.172^{abc}	45.007 ± 0.346^{ab}
3	3	0.426 ± 0.006^{ab}	49.299 ± 1.091^{bc}	0.281 ± 0.020^{a}	5.015 ± 0.048^{ab}	44.979 ± 1.081^{ab}
4	1	0.416 ± 0.009^{ab}	48.377 ± 1.437^{bc}	0.296 ± 0.012^{a}	5.416 ± 0.053^{abc}	45.495 ± 1.375^{ab}
4	2	0.407 ± 0.008^{a}	48.760 ± 0.429^{bc}	0.320 ± 0.013^{a}	5.323 ± 0.014^{abc}	45.191 ± 0.424^{ab}
4	3	0.416 ± 0.003^{ab}	49.381 ± 0.544^{bc}	0.278 ± 0.004^{a}	5.111 ± 0.087^{abc}	44.815 ± 0.502^{ab}
Polarity		–	+	–	+	–

The results are presented as mean ± SD; Different letter within the same column indicate significant differences ($p < 0.05$), according to Tukey's HSD test. Polarity: '+' = the higher the better criteria, '–' = the lower the better criteria; N – nitrogen content [%]; C – carbon content [%]; S – sulphur content [%]; H – hydrogen content [%]; O – oxygen content [%]; Treatment: 1 – Control; 2 – Level 1; 3 – Level 1 + SS; 4 – Level 2; Year: 1–2015.; 2–2016.; 3–2017.

Table 2
Proximate analysis and LHV for *Miscanthus* samples (d.m.).

Case	Treatment	Year	Moisture (%)	Ash (%)	Cfix (%)	Volatile (%)	LHV (MJ/kg)
1	1	1	23.42 ± 2.788^{b}	3.66 ± 0.208^{d}	4.84 ± 0.694^{abc}	88.72 ± 0.409^{abcd}	16.18 ± 0.476^{a}
2	1	2	10.30 ± 1.516^{a}	2.73 ± 0.169^{bcd}	6.06 ± 0.983^{bcd}	86.91 ± 1.102^{abc}	16.48 ± 0.236^{a}
3	1	3	19.69 ± 0.648^{ab}	1.78 ± 0.293^{ab}	8.43 ± 0.084^{ef}	86.85 ± 0.595^{ab}	16.21 ± 0.477^{a}
4	2	1	26.57 ± 4.400^{b}	3.40 ± 1.031^{cd}	4.35 ± 0.580^{ab}	88.97 ± 0.746^{bcd}	16.54 ± 0.393^{a}
5	2	2	10.34 ± 2.377^{a}	2.49 ± 0.409^{abcd}	6.63 ± 0.998^{cde}	86.28 ± 0.443^{a}	16.79 ± 0.429^{a}
6	2	3	25.68 ± 5.915^{b}	1.39 ± 0.006^{a}	9.14 ± 0.827^{f}	86.29 ± 0.700^{a}	16.80 ± 0.647^{a}
7	3	1	27.84 ± 3.494^{b}	3.25 ± 0.918^{cd}	3.85 ± 0.524^{a}	88.81 ± 0.283^{bcd}	16.93 ± 1.238^{a}
8	3	2	11.32 ± 4.251^{a}	2.37 ± 0.189^{abcd}	5.12 ± 1.160^{abc}	87.24 ± 1.418^{abc}	16.88 ± 0.151^{a}
9	3	3	20.33 ± 2.693^{ab}	1.35 ± 0.058^{a}	7.99 ± 0.251^{def}	88.37 ± 0.689^{abcd}	16.39 ± 0.328^{a}
10	4	1	21.77 ± 0.241^{b}	2.44 ± 0.315^{abcd}	5.06 ± 0.045^{abc}	89.33 ± 0.648^{cd}	16.51 ± 0.107^{a}
11	4	2	10.84 ± 1.754^{a}	2.14 ± 0.104^{abcd}	6.12 ± 0.651^{bcd}	87.27 ± 0.891^{abc}	16.41 ± 0.296^{a}
12	4	3	26.64 ± 5.944^{b}	1.57 ± 0.221^{ab}	4.79 ± 0.848^{abc}	90.39 ± 1.246^{d}	16.24 ± 0.256^{a}
	Polarity		–	–	+	/	+

The results are presented as mean ± SD; Different letter within the same column indicate significant differences ($p < 0.05$), according to Tukey's HSD test. Polarity for combustion process: '+' = the higher the better criteria, '–' = the lower the better criteria; Moisture – moisture content [%]; Ash – ash content [%]; Cfix – fixed carbon content [%]; Volatile – volatile matter [%]; LHV – lower heat value [MJ/kg]; Treatment: 1 – Control; 2 – Level 1; 3 – Level 1 + SS; 4 – Level 2.; Year: 1–2015.; 2–2016.; 3–2017.

Table 4
Minor and major elements contents for *Miscanthus x giganteus* samples (d.m.).

Treatment	Year	Cd (mg/kg)	Hg (mg/kg)	Na (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mn (mg/kg)	Cr (mg/kg)
1	1	0.045 ± 0.001^{a}	0.009 ± 0.000^{a}	37.5 ± 6.236^{b}	697 ± 97.208^{ab}	2761 ± 413.102^{f}	100.7 ± 8.437^{d}	10.08 ± 0.725^{bcd}
1	2	0.221 ± 0.051^{a}	0.018 ± 0.001^{a}	23.3 ± 11.282^{ab}	644 ± 53.851^{a}	1467 ± 251.448^{bc}	66.4 ± 23.632^{bc}	5.37 ± 0.196^{a}
1	3	0.121 ± 0.043^{a}	0.013 ± 0.003^{a}	21.0 ± 4.885^{ab}	749 ± 14.566^{ab}	858 ± 221.586^{ab}	37.1 ± 8.818^{ab}	14.83 ± 0.515^{ef}
2	1	0.049 ± 0.003^{a}	0.009 ± 0.000^{a}	35.3 ± 6.873^{ab}	780 ± 34.946^{b}	2655 ± 291.094^{f}	80.0 ± 20.245^{cd}	6.18 ± 0.280^{a}
2	2	2.354 ± 0.490^{b}	0.016 ± 0.002^{a}	20.2 ± 1.539^{ab}	644 ± 37.696^{a}	1321 ± 199.480^{abc}	37.7 ± 5.413^{ab}	6.40 ± 0.758^{ab}
2	3	1.685 ± 0.366^{ab}	0.013 ± 0.001^{a}	21.3 ± 6.286^{ab}	638 ± 57.756^{a}	988 ± 278.059^{ab}	41.9 ± 6.855^{ab}	12.30 ± 1.171^{de}
3	1	0.068 ± 0.005^{a}	0.011 ± 0.000^{a}	31.2 ± 7.763^{ab}	798 ± 31.690^{b}	2411 ± 234.585^{def}	61.5 ± 5.865^{abc}	4.60 ± 0.504^{a}
3	2	2.730 ± 0.236^{b}	0.023 ± 0.005^{a}	28.8 ± 9.927^{ab}	686 ± 38.511^{ab}	1723 ± 235.635^{cd}	62.0 ± 7.156^{abc}	7.08 ± 1.374^{abc}
3	3	1.951 ± 0.217^{ab}	0.009 ± 0.001^{a}	27.3 ± 3.303^{ab}	708 ± 32.929^{ab}	703 ± 12.708^{a}	29.8 ± 7.146^{a}	15.20 ± 2.599^{ef}
4	1	0.069 ± 0.006^{a}	0.011 ± 0.000^{a}	17.1 ± 5.400^{a}	804 ± 25.721^{b}	2504 ± 54.684^{ef}	65.5 ± 7.420^{bc}	6.20 ± 0.648^{a}
4	2	6.758 ± 2.133^{c}	0.109 ± 0.049^{b}	22.90 ± 1.788^{ab}	644 ± 47.448^{a}	1855 ± 312.852^{cde}	58.8 ± 7.469^{abc}	10.79 ± 2.888^{cd}
4	3	3.277 ± 0.044^{b}	0.024 ± 0.017^{a}	22.9 ± 5.660^{ab}	699 ± 8.350^{ab}	1304 ± 28.000^{abc}	56.9 ± 5.440^{abc}	16.87 ± 0.275^{f}
Polarity		–	–	/	–	/	–	–

The results are presented as mean ± SD; Different letter within the same column indicate significant differences ($p < 0.05$), according to Tukey's HSD test. Polarity: '+' = the higher the better criteria, '−' = the lower the better criteria; Treatment: 1 – Control; 2 – Level 1; 3 – Level 1 + SS; 4 – Level 2.

$$Y_k = \beta_{k0} + \sum_{i=1}^{2} \beta_{ki} \cdot X_i + \sum_{i=1}^{2} \beta_{kii} \cdot X_i^2 + \beta_{k12} \cdot X_1 \cdot X_2,\ k = 1 - 17 \quad (1)$$

where: β_{k0}, β_{ki}, β_{kii}, β_{kij} were constant regression coefficients; Y_k, either: Moisture, Ash, Cfix, Volatile, LHV, N, C, S, H, O, Na, K, Ca, Mn, Cr, Cd and Hg, while X1 was applied treatment (Treat.) and X2 was the year of breeding (Year). Further, principal component analysis (PCA) was applied successfully to classify and discriminate between the different juice samples. PCA was applied within the results' descriptors in order to characterize and differentiate between all samples. The assessment of PCA and ANOVA analysis of the acquired outcomes was performed using Statistica software version 12 [56].

3. Results and discussion

3.1. Proximate analysis

The proximate analysis showing: moisture, ash, fixed carbon and volatile matter contents, and lower heat value of *Miscanthus* biomass are shown in Table 2. According to Tukey's HSD in most cases statistically significant differences of all investigated parameters have been found.

Moisture content is the basic parameter that determines the net energy content of biomass material. At the same time, it has a significant influence on the calorific value and combustion process [57,58]. In addition to difficult combustion, the higher moisture content causes problems in the biomass transport as well as increased harmful gases emissions during the combustion process [59]. Depending on the further biomass use/conversion, *Miscanthus* can be harvested from the late autumn to the next spring, when the moisture content is expected to be below 20% [60]. Accordingly, the polarity shown for the moisture content of biomass is defined as (−), although it is important to note that too dry biomass (>10%) can cause problems in pellet production and handling. In addition to the above-mentioned harvest season, relatively wider differences can be expected given the fact that dry matter content is largely influenced by climate conditions of the location where the crop is cultivated. In this investigation determined moisture contents were in the range between 10.30% (II year, I treatment) and 27.84% (I year, III treatment). Moisture content of *Miscanthus* biomass yielded at the spring harvest was 18.0% [61] ie. 25.5% [62], which is partly consistent with the obtained results. CEN/TS 14961 norm [63] does not represent the expected values for moisture content.

Beside moisture content, ash content is also important properties of solid biomass and it is referred to the non-combustible content of biomass [58]. Ash is an undesirable parameter in the biomass because of its catalytic influence on thermal decomposition [64], and its polarity is defined as (−). The importance of the ash content is especially evident in the energy utilization of contaminated biomass, since it is a potentially contaminated material. Generally, agricultural crops/residues, especially grasses, tend to have a higher ash content [64]. In this study, the lowest determined ash content was 1.35% (III year, III treatment), while the highest analyzed content was 3.66% (I year, I treatment). Since Baxter et al. [65] found a higher presence of ash in the *Miscanthus* leaf compared to the stem, the higher proportion of ash in the first year of research may be related to the biomass yields shown in Zgorelec et al. [33]. In that paper, which is also the starting point of the results presented in this study, it can be seen that in the first year of the study compared to the third year *Miscanthus* had have a significantly higher yield of dry matter, which affected the higher share of leaf biomass. Mantineo [66], Meehan et al. [67] and Tran et al. [42], have determined that ash content in *Miscanthus* biomass was respectively: 1.62%, 3.80% and 6.50%. Based on the CEN/TS 14961:2005 solid biofuels differences in ash content in *Miscanthus* biomass could be expected to be between 1% and 6%. In parallel with the results obtained in this study, the presented ash content is in accordance with the above data.

The fix carbon is the quantity of carbon bonded in biomass by the photosynthetic process and represents the mass content of remains after volatiles release, excluding ash and moisture. The higher portion of fix carbon leads to higher quality of biomass because of its positive influence on the heating value [68] and the polarity for the mentioned parameter is defined as (+). Based on the presented values, it can be seen that the content of fixed carbon ranged from 3.85% (year I, treatment III) to 9.14% (year III, treatment II). According to the literature data it can be seen that different contents of fix carbon in *Miscanthus* biomass were determined: 9.5% [69], 11.40% [70], 14.0% [71], 16.0% [72] which are higher values than obtained in this paper. CEN/TS 14961:2005 [63] does not state the expected values for fix carbon.

The volatile matter represents the gaseous phase formed from the thermal degradation of the biomass and its makes biomass easy to ignite [73], which leads to favor combustion reactions [74]. Jenkins et al. [75] and Wilk and Magdziarz [76] noted that high volatile matter indicates a potential for creating large amounts of pollutants emission during ignition process. At the same time higher volatile matter content decrease energy efficiency in case when biomass is directly combusted [77]. Based on the specified volatile matter is defined according to the set polarity as (/). Generally, the volatile matter content is naturally high for many types of biomass [73], which mainly refers to the biomass of agricultural origin. This is confirmed by this study, given the determined content of volatile matter 86.28% (II treatment, II year) - 90.39% (IV treatment, III year). Based on the literature review, the volatile matter in *Miscanthus* biomass was in the range between 72.6% and 87.2% [64,69,78] which is partly consistent with the obtained results. As in the case of moisture content and fixed carbon, CEN/TS 14961 [63] does not state the expected value for volatile content.

The use of biomass as a fuel in thermal and electrical applications requires knowledge of its heating value [38]. Lower heating value (LHV) is the appropriate value to use for the energy available for subsequent

use [71]. Also, it is defined as one of the most important thermo-physical parameters used in the assessment of biomass energy potential [79]. Statistical analyses of LHV showed no significant differences from the investigated parameters, and the established average value was 16,53 MJ/kg. According to the literature data reported LHV values are 15.35 MJ/kg [80], 16.47 MJ/kg [81] and 17.50 MJ/kg [82]. In comparison to a typical variation, LHV value for *Miscanthus* in CEN/TS 14961:2005 [63] specification is ranged between 16 MJ/kg and 19 MJ/kg. In parallel with the results obtained in this study, the presented LHV content is in accordance with the above data.

3.2. Ultimate analysis

The ultimate analysis of *Miscanthus*, presenting: nitrogen, carbon, sulphur, hydrogen and oxygen content was presented in Table 3. Statistical analyses indicated significant differences in most interactions.

Based on ultimate analysis solids biofuels consist mainly of C, O and H. Set polarities C (+), H (+) and O (−) are based on their influence on LHV, considering that higher content of C and H increased heating value, while higher oxygen content decreasing it [39,57,83]. At the same time, C and O react during combustion by generating CO_2 and H_2O [84]. Analyzing the samples in this study, the presented values for C, H and O ranged: 46.34% (II treatment, II year) - 49.91% (I treatment, I year), 5.02% (III treatment, III year) - 5.68% (I treatment, I year), and 44.82% (IV treatment, IV year) - 47.71% (II year, II treatment), respectively. The literature data indicate that values for C content is 46, 75% [67], 43.70% [69], and 49.80% [72], which is consistent with the obtained results. For H content determined values were 4,80% [81]; 5, 80% [67]; 7.32% [85] and for O values were 44.20% Werle et al., [85]; Collura et al. [86], 46.80%; Osman et al. [80], 50.01%, which is consistent with the obtained results. Further, by ISO 17225–1:2014 norm [87] typical variation are ranged between 46% and 52% (C), 5%–6.5% (H) and 40–45% (O) which was also obtained by this research.

Sulphur oxides (SOx) is formatted during combustion and significantly contribute to particulate matter (PM) pollution and acid rain [58]. Beside mainly SO_2 emissions, the S contained in the solid biofuel have also a significant role in corrosion processes [57] and the polarity for S is defined as (−). Of all investigated ultimate analyzes, only the proportion of S showed no significant differences by Tukey's HSD test, and the average value determined was 0.304%. ISO 17225–1:2014 norm specify typical variation between 0.02% and 0.6%. Determined literature values for sulphur content are 0,06% [86], 0.1% [80], 0.2% [69]. In parallel with the results obtained in this study, the presented S content is in accordance with the above data.

Fuel-bound nitrogen causes most of the NOx emissions produced from biomass combustion [58]. An increased content of N in biomass usually results in an increase in NO_X emissions during the combustion process [88] and for this reason, the polarity of N in this study is defined as (−). In addition to other benefits of using biomass in modern boilers, Saidur et al. [89] state the possibility of reducing significant amounts of NO_X emissions, which in some cases can be up to 95% [90]. In this study N content was in the range 0.404% (II treatment, II year) - 0.439% (III treatment, II year). Wilk and Magdzriaz [76], Howell et al. [72] and Osman et al. [80] determined that N content respectively amounted 0, 36%, 0,40% and 1.21%. ISO 17225–1:2014 norm specify typical variation between 0.1% and 1.5%. In parallel with the results obtained in this study, the presented N content is in accordance with the above data.

The chemical analysis of minor and major elements contents of *Miscanthus* has been presented in Table 4, shoving the contents of: Na, K, Ca, Mn, Cr, Cd and Hg. Statistical analyses indicated significant differences in most interactions.

Of the indicated elements in Table 4. Cd and Hg are considered as highly toxic heavy metals [12], they are also characterized by high volatility during the combustion process. High volatility is particularly emphasized Hg and it can be emitted almost totally as vapor [90], while Cd is usually contained in the aerosol fraction [90]. Nzihou and Stanmore [91] state that Cd and Hg are found in fine particles of fly ash, and that use in combustion chambers with effective particulate removal has minimal impact on the atmospheric contamination. Furthermore, the same authors conclude that Hg is the only heavy metal that is not retained in the ash after biomass energy utilization. Since Cd and Hg are defined as highly toxic elements, their polarity is determined as (−). In this study analyzed content of Cd and Hg are raged between: 0.045 mg/kg (I treatment, I year) - 6.758 mg/kg (IV treatment, II year) and 0.009 mg/kg (I, II, III treatment, I, III year) - 0.109 mg/kg (IV treatment, II year), respectively. The content of Cd and Hg in the aboveground *Miscanthus* biomass are primarily related to the concentration in which these elements are present in the soil [27,92], and typical variation on uncontaminated soil are ranged between 0.05 and 0.2 mg/kg (Cd) and <0,02–0,1 mg/kg (Hg) [87]. Obtained results for Cd content partly consistent with the mentioned standard, while results for Hg in accordance with ISO 17225–1:2014 standard [87].

The ash-forming elements (with S and Cl) are especially important for combustion conversion process [93]. Although a certain content of K, Na, Ca volatiles during the combustion process, these elements can be defined as major ash elements, and Cr and Mn as non-volatile minor elements. K affects the occurrence of fouling, corrosion and decrease the ash melting point and it is desirable to lower its content, which is also the case for the content of Na which affects the corrosion mechanisms. In contrast, the same cannot be said for the Ca content since it is defined as an element that improved ash melting behavior [90,94]. Based on the above, certain polarities for the mentioned ash-forming elements are K (−), Na (−) and Ca (+). Based on the performed analyzes, the determined values can be seen for: K 638 mg/kg (II treatment, III year) – 804 mg/kg (IV treatment, I year), Na 17.1 mg/kg (IV treatment, I year) – 35.3 mg/kg (II treatment, I year) and Ca 703 mg/kg (III treatment, III year) - 2761 mg/kg (I treatment, I year). Some of the ash-forming elements were studied by Porbatzki et al. [95] are Monti et al. [96]. They obtained K, Na and Ca values of 3265–7200 mg/kg, 193–280 mg/kg and 3400–5296 mg/kg respectively, which are higher values than obtained in this paper. According to the norm used for solid biofuels typically variations are ranged K (1000 mg/kg – 11 000 mg/kg), Na (20 mg/kg – 100 mg/kg) and Ca (900 mg/kg – 3000 mg/kg) which is partly consistent with the obtained results.

Due to the toxicity/pollution they cause in the agro ecological system, the polarity for Cr and Mn is defined as (−). The content of Cr and Mn in the investigated samples ranged from 4.60 mg/kg (III treatment, I year) – 16.87 mg/kg (IV treatment, III year) and 29.8 mg/kg (III treatment, III year) – 100.7 mg/kg (I treatment, I year), respectively. According to the norm typically variation in *Miscanthus* biomass are ranged between 1 and 6 mg/kg (Cr) and 10–100 mg/kg (Mn), while Mierzwa-Hersztek et al. [79] determined the content of 1.89 mg/kg (Cr) and 34.6 mg/kg (Mn). According to the norm, typically variation in *Miscanthus* biomass is ranged between 1 and 6 mg/kg (Cr) and 10–100 mg/kg (Mn), while Mierzwa-Hersztek et al. [79] determined the content of 1.89 mg/kg (Cr) and 34.6 mg/kg (Mn). In parallel with the results obtained in this study, the presented Mn content is in accordance with the above data while higher Cr content was obtained.

3.3. Principal component analysis (PCA)

The points shown in the PCA graphics, which are geometrically close to each other indicate the similarity of patterns that represent these points. The orientation of the vector describing the variable in factor space indicates an increasing trend of these variables, and the length of the vector is proportional to the square of the correlation values between

the fitting value for the variable and the variable itself. The angles between corresponding variables indicate the degree of their correlations (small angles corresponding to high correlations) [97].

The PCA of the presented data explained that the first three components accounted for 71.95% of the total variance (35.77, 20.82 and 15.36%, respectively) in the seventeen variables system (elements found in the proximate analysis, LHV, elements found in the ultimate analysis and elements found in the chemical analysis of minor and major elements contents). Considering the map of the PCA performed on the data, the contents of ash (which contributed 13.8% of total variance, based on correlations), H (14.3%), Na (8.1%), Ca (14.5%) and Mn (13.2%) exhibited positive scores according to first principal component, whereas Cfix (11.3%) showed a negative score values according to first principal component (Fig. 2). The positive contribution to the second principal component calculation was observed for: LHV (11.3% of total variance, based on correlations) and O (18.9%), while negative scores on second principal component calculation was observed for the content of moisture (9.2%), volatile matter content (9.8%), C content (22.2%) and Cr content (15.8%).

The positive contribution to the third principal component calculation was observed the contents of: S (13.2% of total variance, based on correlations), Cd (12.1%) and Hg (21.8%), while negative scores on second principal component calculation was observed for the content of moisture (9.9%), LHV (7.1%) and K content (12.4%).

3.4. The analysis of variance (ANOVA)

The analysis of variance, exhibited the effects of independent

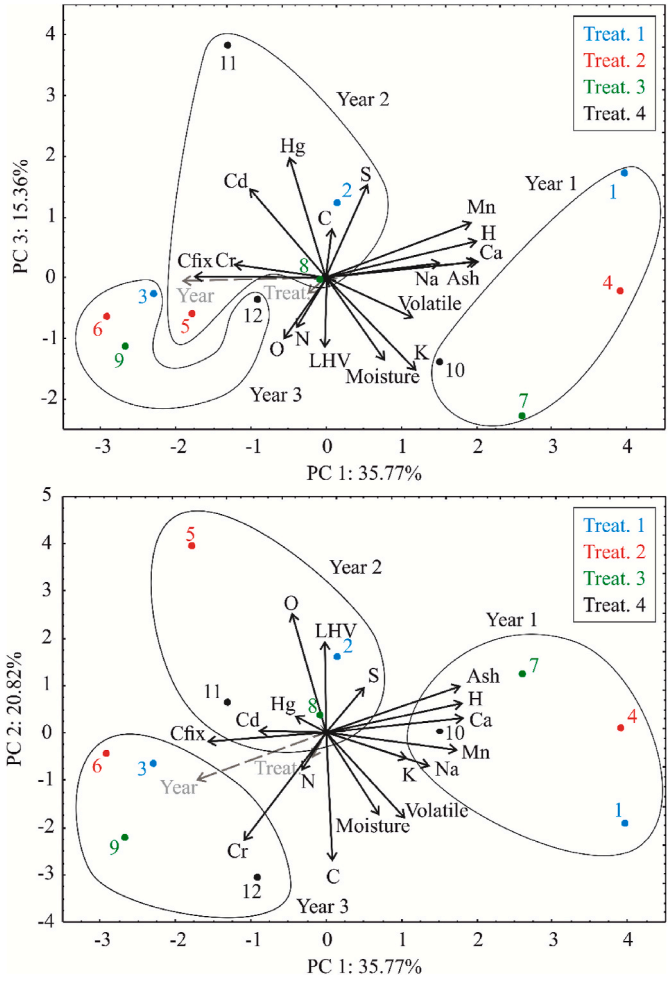

Fig. 2. PCA biplot showing the different samples.

Table 5
The analysis of variance (ANOVA).

	df	Moisture	Ash	Cfix	Volatile	LHV	N	C	S	H	O	Na	K	Ca	Mn	Cr	Cd	Hg
Treat.	1	3.430	0.680^{+}	2.913	4.472*	0.015	0.000	0.007	0.001*	0.007	0.001	35.872	1470.975	42981.914	94.435	2.719	14.813*	0.002
Treat.^{2}	1	7.407	0.000	0.262	1.031	0.444^{+}	0.000	1.200	0.001**	0.002	1.338	31.536	25.472	74844.608	436.250**	12.867**	0.228	0.001
Year	1	6.577	5.531^{+}	18.739^{+}	1.940**	0.034	0.000	0.601	0.000	0.503^{+}	0.003	101.888*	10064.258*	5246478.257^{+}	2520.500^{+}	128.713^{+}	5.784	0.000
Year^2	1	471.061^{+}	0.016	0.014	6.346^{+}	0.073	0.000	1.432	0.000	0.002	1.270	22.601	16948.649*	87858.100	22.906	30.298*	11.845*	0.002
Treat × Year	1	9.204	0.248^{+}	3.751	3.029*	0.072	0.000	1.503	0.000	0.042*	2.012	147.699*	4305.279	106664.141	733.535*	12.401**	2.325	0.000
Error	6	42.457	0.203	6.227	2.405	0.135	0.001	4.724	0.001	0.028	5.205	100.834	9092.251	219274.612	558.698	15.993	10.001	0.004
r^2		0.921	0.970	0.805	0.875	0.825	0.259	0.501	0.770	0.952	0.470	0.771	0.783	0.962	0.872	0.921	0.778	0.573

$^{+}$Statistically significant at $p<0.01$ level; *Statistically significant at $p < 0.05$ level; **Statistically significant at $p < 0.10$ level; Moisture – moisture content; Ash – ash content; Cfix – fixed carbon content; Volatile – volatile matter; LHV – lower heat value.

variables as well as interactions of these variables, to show which of responses were significantly affected by the varying treatment combinations (Table 5).

$$\overline{x}_i = \frac{x_i - \min\limits_i x_i}{\max\limits_i x_i - \min\limits_i x_i},\ \forall i \text{ (used for Cfix content, LHV, C and H content)} \quad (2)$$

$$\overline{x}_i = 1 - \frac{x_i - \min\limits_i x_i}{\max\limits_i x_i - \min\limits_i x_i},\ \forall i \text{ (used for moisture, ash, volatile content, N, S, O, Na, K, Mn and Cr content)} \quad (3)$$

The quadratic term of cultivation year was the most significant term in the SOP model of moisture calculation ($p < 0.01$). The linear terms of treatment and cultivation year, as well as the interchange term of Treat × Year were most significant for ash calculation ($p < 0.01$). The linear term of cultivation year was most significant for Cfix ($p < 0.01$). The quadratic term of cultivation year was the most significant term in the SOP model of volatile matter content calculation ($p < 0.01$), while the linear term of treatment and the non-linear term of Treat × Year were also very significant in volatile matter content calculation ($p < 0.05$). The quadratic term of treatment was the most significant term in the SOP model of LHV calculation ($p < 0.01$). The linear term of treatment was the most significant term in the SOP model for sulphur content calculation ($p < 0.05$). The linear term of cultivation year was the most significant term in the SOP model of hydrogen content calculation ($p < 0.01$), while the non-linear term of Treat × Year was also significant ($p < 0.05$). The linear term of cultivation year and the non-linear term of Treat × Year were the most significant term in the SOP model for sodium content calculation ($p < 0.05$). The linear and the quadratic terms of cultivation year were the most significant term in the SOP model for potassium content calculation ($p < 0.05$). The linear term of cultivation year was the most significant term in the SOP model for calcium content calculation ($p < 0.01$). The linear term of cultivation year was the most significant term in the SOP model for manganese content calculation ($p < 0.01$), while the influence of the non-linear term of Treat × Year was also significant ($p < 0.05$).

The linear term of cultivation year was the most significant term in the SOP model for chromium content calculation ($p < 0.01$), while the influence of the quadratic term of cultivation year was also significant ($p < 0.05$). The linear term of treatment and the quadratic term of cultivation year were the most significant term in the SOP model for cadmium content calculation ($p < 0.05$). There were no statistically significant terms in the SOP models for nitrogen, carbon, oxygen and mercury content calculation. All SOP models represent the data satisfactorily.

The ratio of the explained variation to the total variation was defined as the coefficient of determination (r2) and explained by its magnitude [98]. It is also the share of the variability in the response variable, which is calculated with the regression analysis. A high determination coefficient is indicative that the variation was accounted and that the data adjusted properly to the proposed SOP models. The r2 values for moisture, ash, fixed carbon and volatile matter content, LHV, N, C, S, H, O, Na, K, Ca, Mn, Cr, Cd and Hg contents were 0.921; 0.970; 0.805; 0.875; 0.825; 0.259; 0.501; 0.770; 0.952; 0.470; 0.771; 0.783; 0.962; 0.872; 0.921; 0.778 and 0.573, respectively, were relatively satisfying and present the good adjustment of the model to experimental results.

Standard scores of the seventeen response variables was achieved in order to determinated the breeding variables (year of breeding and the treatment), that give the optimal value of response variables. Min-max optimization procedure was used as optimization method, according to Eq. (2) and Eq. (3):

where, x_i was measured parameter, $\overline{x}_i$ - denoted normalized value, $\forall i$ - for each i.

The "higher the better" or the "lower the better" criteria have been used according to the sign in "Polarity" raw in Tables 1–3.

The standard score (SS) is the mathematical function whose maximum would be determined, by summing the normal scores for of the seventeen responses, according to Eqs. (1) and (2). Each response variable has the same weight during the function SS calculation.

The maximum SS function represents the optimal parameters for processing parameters as well as the optimum for response variables. The SS values were calculated using Eqs. (1) and (2) and presented in Table 1. If the value of standard score is close to 1, it presents the tendency of tested processing parameters of being optimal. The obtained optimal SS value was 0.587, which was obtained using treatment 2 in year of breeding 3. The proximate analysis parameters (moisture, ash, Cfix and volatile matter content) of the optimal sample were: 25.677%, 1.398%, 9.139% and 86.285%, respectively, while LHV value was 16.798 MJ/kg. The ultimate analysis parameters (N, C, S, H and O contents) of the optimal sample were: 0.410%, 49.122%, 0.291%, 5.027% and 45.149%, respectively. The contents of minor and major elements in the chemical analysis (Na, K, Ca, Mn, Cr, Cd and Hg) of the optimal sample were: 21.303 mg/kg; 638.433 mg/kg; 988.467 mg/kg; 41.897 mg/kg; 12.257; mg/kg; 1.685 mg/kg and 0.013 mg/kg, respectively.

4. Conclusion

Soil contamination with mercury and cadmium did not significantly affect the investigated combustion properties of *Miscanthus x giganteus* biomass analyzed after the phytoremediation process. All analyzed parameters, except Cd, K, and Cr, are compatible with or have only expected differences from the literature data and/or ISO 17225–1:2014 norm for solid biofuels. The application of sewage sludge as soil amendment also did not significantly affect the composition of contaminated biomass. Given the established higher proportion of Cd, as a highly volatile metal, the produced biomass can be classified as a hazardous material whose use in the combustion process requires the use of modern combustion plants through technical designs that prevent Cd reemissions into the atmosphere. The applied Response Surface Methodology of *Miscanthus x giganteus* data gave accurate results concerning its quality. The obtained models presented good fitting to experimental results and had described them satisfactorily.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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