Article

The Role of Plant Growth Regulators in *Miscanthus × giganteus* Growth on Trace Elements-Contaminated Soils

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Abstract: Soil contamination with trace elements (TEs) is a pressing problem limiting the cultivation of agricultural crops; however, the non-food energy crop *Miscanthus × giganteus* (*M×g*) can be grown on such soil. The effect of a new plant growth regulator (PGR), Kamethur, and conventional Charkor was studied when *M×g* was cultivated in TE-contaminated soils from Všebořice and Chomutov, in the Northern Czech Republic. Kamethur was beneficial for achieving a higher leaves and stem biomass (by 57.1 and 126%, respectively) in the more contaminated Všebořice soil, while Charkor increased only the leaves biomass (49.5%). Analysis of the comprehensive bio-concentration index showed that Charkor decreased stem accumulation of elements essential for plant development (EEs), as well as the potentially toxic (PTEs) elements, by 33.3 and 11.4%, respectively. Kamethur decreased stem accumulation of EEs by 11.4% and increased the accumulation of PTEs by 23.3%. Statistical evaluation of the current results and literature data illustrated the ability of Charkor to reduce the uptake of PTEs, which is critical for converting clean biomass to bioproducts. Further research should confirm the influence of PGRs on the bioparameters and phytoremediation processes of *M×g* at the field plantation level.

Keywords: plant growth regulators; *Miscanthus × giganteus*; soil; trace elements; comparative effect

1. Introduction

Soil contamination with toxic wastes of industrial and agricultural origins is among the key factors negatively affecting plant development. More than 10 million sites of soil contamination have been reported worldwide [1] leading to the deterioration of the quality of agricultural products and decreasing the harvest value [2,3]. The improvement of cultivation technologies toward strengthening the adaptability of plants to the various stress factors caused by soil contamination and ensuring crop productivity is an urgent task nowadays.

Various development processes can be regulated during vegetation, i.e., acceleration or delay of seed germination, interruption of dormancy in perennial woody plants, stimulation or reduction in shoot elongation, induction of flowering and fruiting, reduction or increase of fruit set, acceleration or delay of senescence processes including fruit ripening and defoliation [4–6]. In this respect, the utilisation of plant growth regulators (PGRs)
represented by natural (phytohormones) or synthetic chemical substances, sounds promising [7–9]. According to biochemical action, Rademacher [10] divided PGRs into “typical” compounds related to auxins, gibberellins and inhibitors of gibberellin biosynthesis, cytokinins, abscisic acid, compounds affecting ethylene status, and compounds related to jasmonic acid and “atypical” phytotoxic PGRs. The benefits achieved by utilising PGRs range from facilitating crop management to increasing crop yield and quality, as well as improving storage and shelf life [7,8,10].

Besides traditional PGRs, including phytohormones and antimicrobial compounds, the synthetic low molecular weight heterocyclic compounds represented by derivatives of pyridine, pyrimidine, pyrazole, triazine, oxazole, oxazolopyrimidine, and aliminoid are promoted [11–13]. This group’s advantage is high efficiency at low concentrations and an absence of toxic effects on cells, which ensures environmental safety [14,15].

The molecular mechanisms concerning the influence of synthetic heterocyclic compounds on plant growth are considerably interesting. It has been hypothesised [16–18] that the regulatory effects may be caused by a stimulatory impact on cell elongation, division, and differentiation, which are the basic mechanisms in the formation and development of shoot and root meristems. Synthetic regulators can indirectly influence plant growth via an endogenous pool of phytohormones in plant cells, which has been confirmed in numerous studies [19–21].

PGR Charkor is a complex mixture of Ivin (2,6-Dimethylpyridine-N-oxide), Emistim C, and the synthetic phytohormone analogue-NAA (1-naphthyl acetic acid) (Figure 1a). Charkor’s active substance is a complex of biologically active compounds, including 1.0 g metabolic products of fungi-micromycetes (Emistim C; 0.11%)-saturated and unsaturated fatty acids (C_{14}–C_{28}), polysaccharides, 15 amino acids, analogues of phytohormones of cytokinin and auxin nature; 8.2 g complex of Ivin with NAA (0.90%); 546 g pure C_{2}H_{5}OH (59.4%); 364 g dH_{2}O (39.6%).

![Chemical structure of Charkor (a) and Kamethur (b).](image)

Recently synthesised, PGR Kamethur (6-methyl-2-mercapto-4-hydroxy pyrimidine potassium salt) is a synthetic heterocyclic compound; a derivative of PGR Methyur (6-methyl-2-mercapto-4-hydroxy pyrimidine sodium salt) (Figure 1b) [22]. The application of Kamethur on a field scale has had a positive effect on the development of the most important crops: maize, barley, oats, sorghum, and sugar beet [22–24] by improving plant adaptation to abiotic stress [25]. The regulatory effect of Kamethur was comparable to or sometimes
exceeded the impact of natural and synthetic auxins, cytokinins (Kinetin and BAP), and Ivin [23]. This PGR was tested for microclonal propagation in vitro [23,24]: its incorporation in the concentration range of $10^{-5}$ to $10^{-8}$ M as a component of the nutrient medium Murashige and Skoog accelerated the formation and development of the root system. The molecular processes are of attention: it was revealed [26] that the regulatory action of Kamethur was associated with driving cell elongation, division, and differentiation.

*Miscanthus × giganteus* (*M×g*) is a popular C₄ energy crop showing a high biomass yield [27–31] and immense lignocellulose content [28]. Its cultivation requires less input compared to other energy crops [32] and promotes carbon sequestration potential [33,34]. Furthermore, having a good tolerance to nutrient deficiency, a wide temperature range, and a good ability to cultivate in marginal and contaminated soils, *M×g* has become effective in phytoremediation processes [33–35]. The crop has been successfully utilised as a phytoagent in soil contaminated by TE [36,37], oil products [38], pesticides [39], and a mixture of chemicals [40]. When phytoremediation was supported by soil amendments [41], microbes [42,43] or plant priming [44], the crop demonstrated sufficient growth even on nutrient-poor and marginal soils. In these cases, cultivation of *M×g* has ecological and economic advantages [45]: in addition to remediation potential, the plant shows high carbon sequestration efficiency [33,34,46], improves soil health [47,48] and demonstrates sufficient biomass yield [27–31,49]. Growing Miscanthus as a non-food crop in contaminated soils is not in conflict with food security and limits contaminant entry into the food chain [50]. Miscanthus biomass can be converted to bio-solid, bio-liquid, and bio-gaseous fuels using thermo-chemical or biological methods [51,52]. It can be processed for use in construction materials, geotextiles, pulp, and paper [53–55] via mechanical or chemical-mechanical pulping [49,56].

Since 2018, Miscanthus has been included in the European Union’s “Greening” approach (Regulation (EU) 2017/2393), which will stimulate farmers to cultivate this crop on a bigger scale. In Central and Eastern Europe, the crop shows good potential for bioenergy and bioeconomy [29,57]. Miscanthus biomass is becoming an important source of energy in Ukraine [35], as the country suffers from a lack of fossil fuels and looks for substitution sources. By 2035, the share of renewable energy in the Ukrainian energy balance is expected to be about 25%, with essential input from fast-growing willow (79%) and *M×g* (15%) [58].

In our previous studies [59–61], the commonly used PGRs, i.e., Charkor, Stimpo, and Regoplant were investigated concerning their impact on *M×g* development in TE-contaminated soil. The results showed a positive influence of PGRs on biomass parameters when the crop was grown in soils with good agricultural properties. The influence of Regoplant was greater than Stimpo, and the best results were obtained with combined treatment: soaking rhizomes in a PGR solution before planting and spraying the biomass during vegetation season [59]. However, the application of PGRs did not improve the biomass parameters when the crop was cultivated in nutrient-poor soil. During the multi-year utilisation, Charkor’s influence was stronger in the second vegetation [61]. However, treatment with Stimpo and Regoplant was not as effective, calling for an expansion in the pool of suitable PGRs that could be recommended to promote the growth of *M×g* in contaminated soils. Kamethur showed promising effects for the development of energy crops: sorghum [24], maize, and wheat [23]. It was expected that exploitation of this PGR in the *M×g* production cycle might also have a positive effect. In this regard, the current research had the following goals:

- To study the effect of Kamethur on the *M×g* production cycle during cultivation in the different TE-contaminated soils;
- To compare the influence of Kamethur on the *M×g* phytoremediation process with the commonly used PGR, Charkor;
- To generalise the peculiarities of the influence of Kamethur and Charkor with other popular regulators, Stimpo and Regoplant, previously utilised in the phytoremediation processes with *M×g* in TE-contaminated soil of different origins.
2. Materials and Methods

2.1. Soil Collection

The experimental soil samples were taken from two localities in the Northern Czech Republic. The first soil sample was taken from the suburb of Chomutov (50°27′38.85″ N, 13°23′07.44″ E). This locality is a former post-mining locality, bombed in 1945 and defined currently as slightly contaminated marginal land. The second soil sample was taken from Všebořice, a suburb quarter of Ústí nad Labem (50°42′11.9″ N 13°58′32.1″ E). This locality is a former open brown coal mining site operated from 1958–1980; the site is currently used as a landfill.

Soil sampling was carried out using the standard approach ISO 11464-2006 [62] and DSTU 4287:2004 [63] from a 5 × 5 m testing square; five soil samples were taken at a depth of 0–30 cm. The pre-treatment of the soil samples to be subjected to physicochemical analysis was carried out in accordance with [62]. After sampling, plant materials and stones were manually removed, and the soil samples were dried until constant weight, passed through a sieve (d = 2 mm), and mixed using the envelope method. The agrochemical characteristics of the initial soils collected from Všebořice and Chomutov are presented in Table 1. The content of TEs in the initial soils is presented in Table 2.

Table 1. The agrochemical characteristics of soils in the pot experiment. Different letters indicate a significant difference between the values within one parameter (p < 0.05).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Chomutov</th>
<th>Všebořice</th>
<th>Measuring Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (KCl)</td>
<td>-</td>
<td>5.24 ± 0.17</td>
<td>5.22 ± 0.07</td>
<td>5.11 ± 0.08</td>
</tr>
<tr>
<td>pH (H₂O)</td>
<td>-</td>
<td>5.98 ± 0.07</td>
<td>5.96 ± 0.03</td>
<td>5.96 ± 0.06</td>
</tr>
<tr>
<td>Organic matter</td>
<td>%</td>
<td>2.86 ± 0.22</td>
<td>2.70 ± 0.32</td>
<td>2.70 ± 0.38</td>
</tr>
<tr>
<td>Alkaline</td>
<td>mg kg⁻¹</td>
<td>3.93 × 10³ [67]</td>
<td>108 [68]</td>
<td></td>
</tr>
<tr>
<td>Available N</td>
<td>mg kg⁻¹</td>
<td>28.8 ± 2.12</td>
<td>29.4 ± 0.71</td>
<td>28.8 ± 0.70</td>
</tr>
<tr>
<td>Available P</td>
<td>mg kg⁻¹</td>
<td>125 ± 1.62 c</td>
<td>126 ± 8.61 c</td>
<td>131 ± 3.97 c</td>
</tr>
<tr>
<td>Available Ca</td>
<td>meq/100 g</td>
<td>1.925 ± 201</td>
<td>1.831 ± 118</td>
<td>1.901 ± 113</td>
</tr>
<tr>
<td>Available Mg</td>
<td>meq/100 g</td>
<td>200 ± 13.5</td>
<td>189 ± 14.6</td>
<td>198 ± 11.2</td>
</tr>
<tr>
<td>Available S</td>
<td>mg kg⁻¹</td>
<td>66.9 ± 1.93</td>
<td>66.7 ± 4.16</td>
<td>68.6 ± 4.85</td>
</tr>
</tbody>
</table>

Table 2. TEs' concentrations (mg kg⁻¹) in the soils from Chomutov and Všebořice; depth of soil sampling: 0–30 cm. Different letters indicate a significant difference between the values within one TE (p < 0.05).

<table>
<thead>
<tr>
<th>TEs</th>
<th>MPC EU [73]</th>
<th>Chomutov</th>
<th>Všebořice</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>13,374 ± 160 a</td>
<td>10,279 ± 1317 b</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Al</td>
<td>96,302 ± 1 018 b</td>
<td>122,530 ± 790 a</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Si</td>
<td>307,255 ± 1 923 a</td>
<td>275,918 ± 783 b</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>1782 ± 152</td>
<td>1936 ± 403</td>
<td>0.568</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>298 ± 15.0  b</td>
<td>1387 ± 42.0 a</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>30,329 ± 688 a</td>
<td>14,828 ± 1947 b</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>7 081 ± 318</td>
<td>8536 ± 1000</td>
<td>0.074</td>
<td></td>
</tr>
<tr>
<td>Ti</td>
<td>7361 ± 119 b</td>
<td>20,489 ± 425 a</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>1500</td>
<td>1478 ± 173 a</td>
<td>546 ± 60.5 b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fe</td>
<td>50,482 ± 864 b</td>
<td>62,150 ± 267 a</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>60</td>
<td>44.6 ± 1.90 b</td>
<td>69.9 ± 13.8 a</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Zn</td>
<td>120</td>
<td>200 ± 8.35</td>
<td>202 ± 11.4</td>
<td>0.023</td>
</tr>
<tr>
<td>Rb</td>
<td>218 ± 9.14 a</td>
<td>122 ± 3.38 b</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Sr</td>
<td>200 [74]</td>
<td>184 ± 11.1 b</td>
<td>410 ± 5.12 a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Zr</td>
<td>343 ± 37.3 b</td>
<td>563 ± 6.31 a</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>60</td>
<td>121 ± 1.45 a</td>
<td>62.2 ± 6.71 b</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The initial soil was stored at 4 °C until used in the pot experiment.
2.2. Experimental Design

The planting material was *Miscanthus × giganteus* J.M. Greef and Deuter ex Hodkinson (*M × g*). Two-year old rhizomes were received from Miscanthus Group, d.o.o, Croatia. A single rhizome containing at least two buds was soaked in PGR solution. Two PGRs were tested in the experiment: Kamethur—a newly synthesised PGR and Charkor—a commonly used PGR.

The treatment solution for Charkor was 0.25%, based on the previous investigation [75]; the treatment solution for Kamethur was 1%, based on the recommendation in [22]. The exposition time for both PGRs was 24 h; for the control experiment, distilled water was used instead of the PGR solution.

The preparation of the PGR solutions and the layout of treatment were explained in detail in [59]. At the end of the exposure time, the individual rhizome was removed from the suspension, soaked with filter paper, and immediately planted in the pot. In each pot, 1 kg of keramzite (drainage) was added to the bottom, followed by 5 kg of the research soil. Each variant of the experiment was provided in three parallel variants, consequently, there were 9 monitored pots for each soil, i.e., the Všebořice and Chomutov soils.

The pots with the planted rhizomes were kept outside under natural conditions till harvest. The soil was irrigated with tap water at regular intervals to maintain soil moisture. The biological parameters: plant height, tillers quantity, number of leaves, leaf length and width, were monitored monthly at equal intervals. The total area of one plant was calculated based on the leaf surface area (*LSA*; *leaf length* and *width*) and the number of leaves, according to the following formulas:

\[
LSA = \text{Leaf length} \times \text{Leaf width} \times 0.67 \\
Plant \text{ total area} = LSA \times LQ
\]

where \(LQ\)—leaves quantity per plant.

The pot experiment started on 22 April 2021 and finished on 19 October 2021, when the plants became yellow.

2.3. Samples Collection at Harvest

*M × g* aboveground biomass (AGB) was harvested at the end of the vegetation period. The samples were collected following the standard: DSTU 4287:2004 [63] and ISO 11464:2007 [62]. The AGB was dried till a constant weight in the open air. The dry biomass weight (DW) value was calculated for the leaves and stems separately. Samples of leaves and stems were separately collected into labelled plastic zip-lock bags and stored at room temperature until the chemical analysis. The roots were left in the pots to be monitored for further years.

2.4. Analysis of TEs’ Content in the Soil and Biomass

The preparation of the soil samples for analysis was performed according to ISO 11464:2006 [62]. Briefly, the soil sample was dried at 105 °C to a constant weight. The dry sample was put on a clean sheet of paper, and small stones, plant particles, and other inclusions were removed. Next, bigger soil clods were ground in a porcelain mortar and mixed with the main part of the soil sample. Then, thoroughly mixed soil was put onto clean paper in a square and divided into four equal parts with a spatula. Two opposite parts were removed, and the two others were combined, remixed, and taken for further analysis. This average sample was additionally sieved (0.25 mm pore size). Finally, the bigger particles were milled if necessary [62].

The preparation of the stem and leaf samples for analysis was performed following the standard DSTU ISO 11465-2001 [76]. Samples were dried at 105 °C to a constant weight, cooled in desiccators for 1 h, and weighed.

The estimation of TEs’ content in the soil and plant samples was carried out using X-ray fluorescence analysis following the United States Environmental Protection Agency
standard [77] using an Elvax Light SDD Analyzer, Elvatech, Kyiv, Ukraine. The device can detect elements in a range of $^{11}$Na to $^{92}$U with high accuracy (0.01%). The time of the data collection was $2 \times 180$ s; the limits of absolute measuring error were $\pm 0.05$–$0.2\%$ (with the time for one measurement equal to $180$ s). The layout of the analysis was described in detail earlier [59]. The plant samples were combusted for 4 h at $400$ °C, cooled for 1 h in desiccators, weighed, and processed for TE analysis. Three parallel measurements were taken for each sample. The level of TEs in the soil was determined in mg kg$^{-1}$. The level of TEs in the biomass was determined in mass units in the ash and then recalculated to mg kg$^{-1}$ based on the ash content of the initial plant material. For the overall calculation, the concentration was expressed in mg kg$^{-1}$ dry weight. In the case of the soil analysis, the samples (~2 g) were placed on ultra-thin (4 μm) polypropylene film (supplied with the device), which is transparent to X-rays, and further accurately transferred to the device where the measurement was performed. In the case of biomass, the combusted samples (ash) of the stems and leaves (~0.5 g) were placed inside a plastic ring with a diameter of 1.25 cm, which was located on a similar thin polypropylene film, and compacted using a glass rod. The resulting sample was transferred into a device for measurement [59].

2.5. Calculation of Phytoremediation Parameters

The bioconcentration factor (BCF) is the ratio between the contaminant’s concentration in the plant tissue and its concentration in the soil. The coefficient was calculated according to Zayed et al. [78]:

$$BCF = \frac{\text{Contaminant concentration in plant tissues (mg kg}^{-1}\text{) at harvest}}{\text{Initial contaminant concentration in soil (mg kg}^{-1}\text{)}}$$

(3)

The comprehensive bio-concentration index (CBCI) is a predictive index used to assess the ability of different phytoagents to accumulate multiple TEs. The index was calculated using the following equation [79]:

$$\text{CBCI} = \frac{1}{n} \sum_{i=1}^{n} \frac{BCF_i - BCF_{i,\text{min}}}{BCF_{i,\text{max}} - BCF_{i,\text{min}}}$$

(4)

where $n$ is the total number of TEs, and $i$ is a particular TE.

2.6. Statistical Evaluation

Statistical data analysis was performed using RStudio software (version 2022.07.2+576 “Spotted Wakerobin”, RStudio PBC, 2022). A two-way repeated measures analysis of variance (RM ANOVA) was performed to detect statistically significant differences in growth dynamics, the number of shoots, stem diameter, and total plant area, between different treatments (at three levels), taking into account the soil contamination (at two levels) within specific time points. The significance of differences in the aboveground biomass ($M \times g$ DW) and the values of the BCF considering the soil contamination, PGR treatment, and the plant part was determined using a Three-Way ANOVA. In cases where a significant difference was detected by ANOVA, Tukey’s HSD test was performed for pairwise comparison. The treatments were categorised according to the results of this test (by letters in descending order), and boxplots and graphs were produced.

3. Results and Discussion

3.1. Influence of PGRs and Soil Contamination on $M \times g$ Parameters

The interconnection between the contamination level (soil) and the PGRs (treatment) during the $M \times g$ vegetation (time) is presented in Table S1. The results showed the absence of a cumulative effect on the plant height ($p = 0.25$). At the beginning of the vegetation period, the PGRs affected the plant height; thereafter, the effect was less visible (Figure 2). In the Všebořice soil, the PGRs increased the height, while in the Chomutov soil, they decreased it (Figure 2).
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![Figure 2. The growth dynamic of $M \times g$ during cultivation in Chomutov and Všebořice soils. S–soil; T–treatment. Different letters within one month indicate a significant difference between the values.](image)

PGRs differently impacted the number of shoots (Table S1). At the beginning of the vegetation period (June 2021), Kamethur increased the value by 245% in the Chomutov soil; at the end of the vegetation period (October 2021), the number was the same as for the control (Table S2). In the Všebořice soil, at the beginning of the vegetation period, the number of shoots with Kamethur was equal to the control and decreased by 43.5% at the end (Table S2).

In both soils, the stem’s diameter was not complexly affected by soil contamination, PGRs, or time (Table S1; $p = 0.98$).

At the end of the vegetation period, a cumulative effect of soil contamination and treatment on the total plant area was observed (Figure 3). Treatment with Charkor reduced the total area of the plant in the Chomutov and Všebořice soils by 36.4 and 27.2%, respectively, compared to the control (Figure 3). The Kamethur treatment did not have a statistically significant effect on the total plant area but showed a different effect depending on the soil: Kamethur decreased the total area in the Chomutov soil and increased this parameter in the Všebořice soil (Figure 3).

Individually, the soil contamination did not influence $M \times g$ yield, whereas the cumulative effect of soil contamination and PGR was statistically significant (Table S1). The leaves and stems DW was dependent on soil contamination (Table S1, Figure 4); lower stem DW was observed for the plant in Všebořice soil (Figure 4). Charkor increased the leaf DW in Všebořice soil, while Kamethur increased this parameter in both soils. Treatment with Kamethur increased the stem’s DW in the Všebořice soil (Figure 5).
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Figure 3. The calculated total area of $M \times g$ at harvest. Different letters indicate a significant difference between the values ($p < 0.05$).

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Thus, Kamethur positively affects leaf DW in both soils, and stem DW in more contaminated soil (Všebořice). The influence of Charkor was observed only for leaf DW in Všebořice soil.

3.2. Impact of PGRs on Phytoremediation Parameters

The concentration of the monitored elements in the plants’ tissues is presented in Table S3. The influence of PGRs on the $M \times g$ phytoremediation process was evaluated...
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3.2. Impact of PGRs on Phytoremediation Parameters

The concentration of the monitored elements in the plants’ tissues is presented in Table S3. The influence of PGRs on the M×g phytoremediation process was evaluated based on the bioconcentration factor (BCF) calculated separately for leaves and stems (Table S4). Statistical analysis showed that for S and Mn (Table S4, Figure 5), a cumulative effect of soil contamination and PGRs as a function of the plant’s parts was absent. Some trends were visible for other elements, i.e., (a) BCFs of Si and Fe in stems were not affected by soil contamination and PGRs; (b) BCFs of K and Rb in leaves were not affected by PGRs in Chomutov soil; (c) BCFs of Al, P, and Zn in leaves were not affected by PGRs in Všebořice soil; (d) BCFs of Ca, Ti, and Sr in stems were not affected by PGRs in Všebořice soil (Figure 5).

Kamethur significantly increased the accumulation of Mg, Al, P, Ca, Ti, Fe, Cu, and Pb; both PGRs increased the accumulation of Si and Sr; and Charkor essentially decreased Fe accumulation in leaves in the Chomutov soil. However, in the Všebořice soil (Table S4, Figure 5), the effect of PGRs on the accumulation of TEs in leaves was absent.

PGRs significantly decreased Mg accumulation in stems in both soils, while increased Sr accumulation in the Chomutov soil (Table S4, Figure 5). Treatment by Kamethur in Chomutov soil increased the accumulation of Zn and Rb in stems, while in the Všebořice soil, the accumulation of Zn decreased and the accumulation of K increased (Table S4, Figure 5). In the Všebořice soil, Charkor decreased the accumulation of K and Rb in stems (Figure 5).

Thus, Kamethur did not affect (14 of 15) or decreased the accumulation of TEs in leaves in the Všebořice soil; whereas, it had no effect (9 of 13) and decreased (3 of 13) the accumulation of TEs in stems (Figure 5). In the Chomutov soil, Kamethur increased TE accumulation in leaves (12 of 15) and did not influence TE accumulation in stems (7 of 13) (Figure 5).

Treatment with Charkor did not influence or decrease TE accumulation in leaves and stems in both soils, except for increasing Si accumulation in leaves and Sr accumulation in leaves and stems in the Chomutov soil (Figure 5).

In order to sum up the influence of PGRs on M×g’s phytoremediation potential, a comprehensive bio-concentration index (CBCI) was calculated. This parameter was earlier utilised for energy crops [79], including M×g [61], to estimate the overall ability to accumulate TEs. Analysis was performed for two groups of elements [80,81]. The first group was formed by elements essential for plant development (EES), i.e., Mg, Al, Si, P, S, K, and Ca; the second group included potentially toxic elements (PTEs), which inhibit plant development, i.e., Ti, Mn, Fe, Cu, Zn, Rb, Sr, and Pb.
When \(M \times g\) grew in the Chomutov soil (Figure 6), the control plant accumulated EEs and PTEs mainly in the stems. Under the treatment with PGRs, both elements’ groups were mainly accumulated in the leaves (Figure 6a,b). Charkor increased the accumulation of EEs and PTEs in leaves by 12.4 and 21.2%, respectively; Kamethur resulted in a much greater accumulation of both groups’ elements in the leaves by 93.1 and 69.5%, respectively. Accumulation in stems had a different tendency: Charkor decreased EEs’ accumulation in stems by 33.3 and 11.4%, respectively; Kamethur decreased the accumulation of EEs by 11.4% and increased the accumulation of PTEs by 23.3%.

**Figure 6.** Comprehensive bio-concentration index for: (a) elements essential for the plant’s development, and (b) elements potentially toxic for the plant’s development.

In the Všebořice soil, the control plant accumulated EEs mainly in leaves, while the PTEs mainly accumulated in the stems. Charkor reduced the accumulation of EEs and PTEs in leaves by 31.4 and 4.1%, respectively; and in stems by 17.6 and 43.4%, respectively. Kamethur showed a different effect: it increased EEs’ accumulation in stems by 12.0% and reduced it in leaves by 29%. Kamethur resulted in decreasing PTEs’ accumulation in stems by 21.7% and increasing their accumulation in leaves by 40.6%.

Charkor affected the accumulation of EEs in both soils in a similar way, increasing the accumulation of EEs’ elements in leaves and decreasing them in stems (Figure 6a) PGR application in less contaminated soil (Chomutov) led to a redistribution of EEs in vegetative organs: accumulation in the leaves increased and decreased in the stems. PGR application in more contaminated soil (Všebořice) led to the inhibition of EEs’ accumulation in leaves; the EEs’ accumulation in stems was contradictory and requires additional study.

The PGRs in the Chomutov soil increased the accumulation of PTEs in leaves and differently impacted their accumulation in stems: Charkor decreased PTEs’ accumulation while Kamethur increased it. The PGRs decreased PTEs’ accumulation in stems in Vše-
bořice soil while the PGRs’ impact on their accumulation in leaves was different: Charkor decreased PTEs’ accumulation whereas Kamethur increased this value.

The summed results showed that Charkor reduced PTEs’ accumulation in $M \times g$’s aboveground biomass (leaves and stems). This fact is critical for ensuring the production of clean biomass during $M \times g$ cultivation in TE-contaminated soils of different origins (Figure 6b).

3.3. Comparative Analysis of PGRs Impact on $M \times g$ Biomass Productivity

PCA analysis was performed to summarise the impact of the PGRs on $M \times g$ biomass productivity. The influence of Kamethur and Charkor, researched in the current study, and the impact of the PGRs Stimpo and Regoplant, tested previously [59,75] were compared. The soils where $M \times g$ was cultivated with PGRs had different contamination levels and agrochemical properties (Table 3). There were five TEs (Mn, Cu, Zn, Sr, and Pb) detected in the comparative soils (Chomutov, Všebořice, Dolyna), which were taken into account during the PCA performance. The results are presented in Figure 7 and indicate that the first two components (PC1 and PC2) covered 72.3% of the data, while 50.9% of the data was covered by PC1.

Table 3. TE concentrations (mg kg$^{-1}$) in soils. Different letters indicate a significant difference between the values within one TE ($p < 0.05$).

<table>
<thead>
<tr>
<th>TE</th>
<th>MPC EU [58]</th>
<th>Chomutov</th>
<th>Všebořice</th>
<th>Dolyna [75]</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr</td>
<td>90</td>
<td>-</td>
<td>-</td>
<td>141 ± 4.50</td>
<td>-</td>
</tr>
<tr>
<td>Mn</td>
<td>1500</td>
<td>1 478 ± 173 a</td>
<td>546 ± 60.5 c</td>
<td>933 ± 22.5 b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ni</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>66.0 ± 2.00</td>
<td>-</td>
</tr>
<tr>
<td>Cu</td>
<td>60</td>
<td>44.6 ± 1.90 b</td>
<td>69.9 ± 13.8 a</td>
<td>43.5 ± 0.50 b</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Zn</td>
<td>120</td>
<td>200 ± 8.35 a</td>
<td>202 ± 11.4 a</td>
<td>116 ± 1.00 b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sr</td>
<td>200 [59]</td>
<td>184 ± 11.1 c</td>
<td>202 ± 11.4 a</td>
<td>223 ± 1.50 b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pb</td>
<td>60</td>
<td>121 ± 1.45 a</td>
<td>62.2 ± 6.71 b</td>
<td>15.5 ± 0.50 c</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Figure 7. Biplot of PC1 and PC2 for PGRs, plants, and soil data.
The control plant had a higher number of shoots. Its development was determined by the Ca, Mg, K, and C present in the soil. Charkor and Kamethur increased the M×g tillers quantity, while Regoplant increased the aboveground biomass DW and the uptake of Mn, Cu, Zn, Sr, and Pb into the leaves and stems (Figure 7). However, the application of Regoplant did not have a visible influence on the plant’s bioparameters. A similar observation was reported when Regoplant was applied to another energy crop—sorghum [82], despite earlier results proving the positive effect of Regoplant on corn, barley, and sugar beets [7] by improving yield, and enhancing their tolerance to parasites [83]. Stimpo mainly affected plant height; its influence was dependent on soil pH and phosphate content.

The positive impact of Kamethur on enhancing the biomass of sorghum cultivated in regular agricultural soil was explained by the intensification of the formation of roots and stems accompanied by higher photosynthesis activity [26]. Although, in our study, Kamethur was not so effective (the harvested biomass enrichment was only 57.1 and 126%), this lesser increase may be due to the cultivation of M×g in the contaminated soil. No previous study has examined the influence of Kamethur on the phytoremediation process of energy crops, including M×g, which was carried out in the current research.

The results showed that the soil’s pH and phosphate content affected the plant’s height, while other agrochemical parameters (Ca, Mg, K, and C) influenced the tiller quantity. Other important soil elements, i.e., P, Ca, Mg, K, and C did not significantly influence the DW value, which is in line with earlier published conclusions [84,85] concerning the limited influence of soil mineral fertilisation upon M×g yield. The influence of soil contamination on M×g development was only evident for Cu (Figure 7).

3.4. Comparative Impact of PGR Charkor on M×g Development in the Different Soils

In order to investigate the effect of the common PGR, Charkor, on M×g growth and biomass productivity (Table 3), a comparative analysis of the current results and previously published observations [75] was performed; AGB DW, tillers quantity, and plant height were considered.

The AGB DW value of the control plant was highest in the Chomutov soil, followed by an intermediate value in the Všebořice soil; and the lowest value in the Dolyna soil (Figure 8a). Treatment with Charkor in the Všebořice and Dolyna soils resulted in a significant increase in AGB DW by 56.7 and 113%, respectively, with a slight AGB DW increase in the Chomutov soil (10.2%).

Charkor positively affected M×g height only in the Dolyna soil: the increase was 44.7% (Figure 8b); the effect was negligible in other soils.

![Figure 8.](image-url)
The number of shoots in untreated plants cultivated in the compared soils did not show a significant difference (Figure 8c). Treatment with Charkor positively affected the number of shoots; however, a significant difference was found only in the Chomutov soil (Figure 8c).

4. Conclusions

It can be concluded that the PGR Kamethur is more beneficial for yielding higher biomass during M×g cultivation in the more contaminated Všebořice soil, while Charkor can be used to reduce the uptake of potentially toxic elements (PTEs) to AGB in differently contaminated soils.

The PGR Charkor increased the accumulation of elements essential for plant development (EEs) in leaves and decreased them in stems. The application of PGRs to plants cultivated in more contaminated soil resulted in an inhibition of EEs’ accumulation in leaves, while their accumulation in stems was quite contradictory. When M×g was cultivated in the less contaminated Chomutov soil, the application of PGRs increased the accumulation of PTEs in leaves and differently affected their accumulation in stems: Charkor decreased the accumulation of PTEs and Kamethur increased them. The application of both PGRs in more contaminated soil decreased the accumulation of PTEs in stems; the application of Charkor decreased the accumulation of PTEs in leaves and Kamethur increased them.

To the best of our knowledge, this is the first attempt to examine the influence of the PGR Kamethur on the bioparameters and phytoremediation process of the energy crop M×g. The comparative analysis of current study results and previous findings showed that the PGR Charkor reduced the accumulation of PTEs into M×g’s biomass (leaves and stems), which is critical for ensuring its clean production. This effect has to be confirmed in a field-scale experiment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12122999/s1, Table S1: ANOVA outputs of processing physiological parameters; Table S2: Physiological parameters of M×g at the beginning and the end of the vegetation season. Different letters within one month indicate a significant difference between the values; Table S3: TE concentrations (in mg kg⁻¹) accumulated in M×g leaves and stems after the vegetation season. Different letters within one month indicate a significant difference between the values; Table S4: BCF values for TEs accumulated in M×g leaves and stems after the vegetation season. Different letters within one month indicate a significant difference between the values.
Author Contributions: Conceptualization, V.P.; methodology, A.M. and R.A.N.; software, A.M. and O.Z.; validation, A.M., R.A.N. and P.S.; formal analysis, V.P., A.M. and T.S.; investigation A.M., R.A.N., V.T. and P.S.; resources, V.P.; data curation, A.M., O.Z., R.A.N. and T.S.; writing—original draft preparation, A.M., V.P., T.S. and O.Z.; writing—review and editing, V.P. and A.M.; visualisation, A.M.; supervision, V.P.; project administration, A.M.; funding acquisition, V.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Czech-German project “MiscanValue-Cornet” (Reg. No. CZ.01.1.02/0.0/0.0/19_263/0018837).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is available on request from the corresponding author.

Acknowledgments: The authors would like to thank Ing. Vojtech Vana and Sergey Ust’ak, Crop Research Institute, Branch in Chomutov, the Czech Republic for their assistance in maintaining the experiment.

Conflicts of Interest: The authors declare no conflict of interest.

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