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PHYSIOLOGICAL PARAMETERS AND METAL-ACCUMULATING CAPACITY OF THE BIOFUEL PLANT *MISCANTHUS × GIGANTEUS* CULTIVATED ON OIL-CONTAMINATED PODZOL SOIL AND TREATED WITH HUMIC PREPARATIONS

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Background. Physiological characteristics of the biofuel plant *Miscanthus × giganteus* J. M. Greef, Deuter ex Hodk. & Renvoize are currently attracting much attention due to its phytoremediation potential. The aim of this work was to study the content of photosynthetic pigments in the leaves of *M. giganteus*, the accumulation of metals in the rhizosphere and aboveground organs, as well as the morphological parameters of plants cultivated on oil-contaminated soil and exposed to treatment with humic preparations.

Materials and Methods. During field experiments, five experimental plots (PC and P1–P4) with an area of 1 m^2 were laid out on podzol soil in the territory adjacent to the Starosambirske oil field. The PC plot was not subjected to any experimental treatment. The soil in plot P1 was planted with *M. giganteus* rhizomes; the soils in plots P2–P4 were contaminated with 10 L/m^2 of crude oil and then planted with *M. giganteus* rhizomes. Before planting the rhizomes on plots P3 and P4, these were soaked in solutions of Fulvital® Plus Liquid and Humifield® Forte, respectively. During the growth period, the plants were sprayed twice with humic preparations.

Shoot height and leaf width, *a*- and *b*-type chlorophyll (Chl_a and Chl_b, respectively), total chlorophyll (Chl_{a+b}) and carotenoid concentrations were measured using standard methods. The content of metals (Ca, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb, Zn) in soil and plant samples was assessed by X-ray fluorescence analysis using an Elvax Light SDD Analyzer.

Results. The cultivation of *M. giganteus* on oil-contaminated soil did not affect shoot height or leaf width of plants, but it reduced the content of Chl_a, Chl_b, Chl_{a+b} and carotenoids in plant leaves. Treatment of plants with humic preparations led to an increase in pigment concentrations in the leaves at different growth periods. Oil-contaminated soil planted with *M. giganteus* showed elevated levels of Cr and Ni. The cultivation of *M. giganteus* treated with Fulvital® Plus Liquid resulted in increased Ca, Mn and Ni contents in rhizosphere soil of an oil-contaminated plot. Growing *M. giganteus* on oil-contaminated soil resulted in significant decreases in Ca, Cr, Fe, K, Mg, Ni and Zn concentrations in plant stems. Treatment with humic preparations increased the content of the mentioned metals in the stems and the concentration of Mg and Ni in the leaves of plants from oil-contaminated soil compared to those in untreated plants. According to the bioaccumulation factor (BF) values, *M. giganteus* leaves have a high accumulation potential for Ni and Ca (BF>1), a medium accumulation potential for Mg, K and Cr (BF from 0.1 to 0.32) and a low accumulation potential for Fe and Zn (BF<0.1). The BF values of metals in leaves and stems decreased when plants were grown on oil-contaminated soil.

Conclusions. Humic preparation treatment has a positive effect on the physiological parameters of *M. giganteus* grown on oil-contaminated podzol soil. The ability of *M. giganteus* to extract Ni from soil may mediate the plant's phytoremediation potential. In this regard, the cultivation of *M. giganteus* in combination with its treatment with humic preparations will be promising on lands contaminated with oil and petroleum products.

Keywords: *Miscanthus × giganteus*, biofuel crops, oil-contaminated soil, podzol soils, humic preparations, phytoremediation, heavy metals, photosynthesis

INTRODUCTION

Oil is a key energy resource of strategic importance that affects the economic stability of each country. However, the processes of oil production, transportation and storage are associated with the risk of its leaks and spills leading to environmental contamination and posing a serious threat to natural ecosystems (Jernelöv, 2018; Galieriková & Materna, 2020). This type of pollution is associated mainly with the release of petroleum hydrocarbons and their derivatives, many of which, being persistent in soil and natural waters, exhibit varying levels of toxicity to living systems (Roy et al., 2023). Oil components significantly alter soil properties, affect microbial diversity and hinder plant growth and productivity (Athar *et al.*, 2016; Jernelöv, 2018; da Silva Correa *et al.*, 2022). In addition, crude oil and petroleum products contain a certain amount of heavy metals, which aggravate the negative consequences of oil pollution on the environment (Gan *et al.*, 2022; Aradhi *et al.*, 2023; Singha & Deka, 2024).

Soil contamination with crude oil, petroleum products and waste from the petroleum industry often occurs in oil-producing regions of the world, including those in the territory of Ukraine (Dzura & Podan, 2017; Karabyn *et al.*, 2019; Drozd *et al.*, 2021). As a result, soils adjacent to oil production sites may lose their physicochemical, biological and ecological properties and become susceptible to salinization and erosion (Jernelöv, 2018; Drozd *et al.*, 2021).

Extensive oil-contaminated lands pose a risk to farming and crop production and are potential sources of surface water and groundwater pollution. In this regard, phytoremediation has been recognized as one of the cost-effective and environmentally friendly approaches to the restoration of degraded soils. Phytoremediation techniques are based on the use of plant species that, together with the rhizosphere microbiota, are capable of purifying the contaminated environment from various pollutants, including petroleum hydrocarbons and heavy metals (Wang *et al.*, 2022; Burdová *et al.*, 2023).

At the same time, much attention is currently being paid to the problem of economic use of land massifs with altered and deteriorated soil properties due to pollution with crude oil and petroleum products. In this context, second-generation biofuel crops are of considerable practical interest as candidates for cultivation on degraded lands (Wiens *et al.*, 2011; Tudge *et al.*, 2021). Second-generation biofuel crops are those that are not used for food purposes (non-food crops). Second-generation biofuel crops are able to grow on marginal soils that would otherwise be unsuitable for growing food crops and produce biomass that can be converted into biofuels without competing with food production (Erb *et al.*, 2012). In addition, some biofuel crops can also perform bioremediation functions, improving soil quality and restoring its ecological properties.

One of the promising second generation biofuel plants is the member of the family Poaceae, *Miscanthus* × *giganteus* J. M. Greef, Deuter ex Hodk. & Renvoize (giant miscanthus), a sterile hybrid of *Miscanthus sinensis* and *Miscanthus sacchariflorus*. Giant miscanthus is a highly productive energy crop whose biomass can be used for biofuel production (Lee & Kuan, 2015). This is a tall (3–4 m in height), fast-growing perennial plant with a C-4 photosynthetic pathway that reproduces by underground rhizomes (Beale *et al.*, 1996). The plants have been shown to be stress-resistant and highly adaptable to climate conditions (Bastia *et al.*, 2023; Burdová *et al.*, 2023). Several studies have suggested that *M. giganteus* has a phytoremediation potential (Podan & Dzhura, 2019; Pidlisnyuk *et al.*, 2022; Pysarenko & Bezsonova, 2020; Nsanganwimana *et al.*, 2021; Burdová *et al.*, 2023). However, the physiological processes in *M. giganteus* plants cultivated on oil-contaminated soils have not been sufficiently studied.

Research in recent decades has revealed that application of humic preparations increases the efficiency of agricultural technologies, in particular on lands subjected to degradation (Marenych *et al.*, 2019; Borzykh *et al.*, 2023). Modern preparations prepared on the basis of humic substances also contain macro- and micronutrients, amino acids and biologically active compounds; these act as growth stimulants and increase plant resistance to adverse environmental conditions and diseases (Borzykh *et al.*, 2023). Previous research has shown that growing *M. giganteus* treated with humic preparations on oil-contaminated soil improves soil properties by regulating its acid-base balance and increasing humus content (Podan & Dzhura, 2019).

The aim of this work was to study the morphological parameters of *M. giganteus*, the concentration of photosynthetic pigments in plant leaves and the accumulation of metals in the rhizosphere and aboveground organs of plants under conditions of soil contamination with crude oil and treatment of plants with humic preparations.

MATERIALS AND METHODS

Experimental design. Field experiments were conducted from 2018 to 2022 in the territory adjacent to the Starosambirske oil field in the western part of Ukraine, on podzol soil. The soil pH value was in the range of 4.10–4.20 (Podan & Dzura, 2019). According to the type of soil formation, the soils of the study area can be classified as sod podzolized gleyed ones (Pozniak, 2019).

Five experimental plots (PC and P1–P4) with an area of 1 m^2 each were laid out in the study territory (**Table 1**). The PC plot was not subjected to any experimental treatment and served as a control when studying the concentration of metals in the soil. The soil on the plot P1 was planted with the rhizomes of *M. giganteus*; the soils on three other plots (P2–P4) were contaminated with crude oil at 10 L/m^2 and then planted with *M. giganteus* rhizomes. The period between the contamination of the soil with crude oil and the planting of the *M. giganteus* rhizomes was about six months.

Before planting on plots P3 and P4, the rhizomes were soaked in solutions of Fulvital® Plus Liquid and Humifield® Forte (Humintech GmbH, Germany), respectively. The concentration of the preparations was 0.2 g per 1 L of water. During the growing season, the aerial parts of plants cultivated on plots P3 and P4 were sprayed twice with the above-mentioned preparations.

Table 1. **Variations of the study**

Plot	Experimental conditions
PC	Uncontaminated soil (control)
P1	Uncontaminated soil planted with *M. giganteus*
P2	Oil-contaminated soil planted with *M. giganteus*
P3	Oil-contaminated soil with *M. giganteus* plants treated with Fulvital® Plus Liquid* (rhizome soaking and plant spraying)
P4	Oil-contaminated soil with *M. giganteus* plants treated with Humifield® Forte** (rhizome soaking and plant spraying)

Notes: *Fulvital® Plus Liquid (hereinafter referred to as Fulvital) is a water-soluble, low-molecular plant growth stimulant that improves nutrient uptake by plants and reduces nutrient immobilization in the soil; in addition, it supplies Fe, Zn, Mn and Cu to promote plant development;
**Humifield® Forte (hereinafter referred to as Humifield) is an anti-stress preparation and plant growth stimulant containing salts of fulvic and humic acids, amino acids, potassium and trace elements

Plots were planted in March and plant material samples were collected in July and September for the evaluation of photosynthetic pigment concentrations and morphological parameters (shoot height and leaf width) using standard methods. In September (after six months of *M. giganteus* growth), soil samples from plots PC, P2–P4 and plant material from plots P1–P4 were collected for the evaluation of metal concentrations.

Analysis of metal concentrations. Soil samples from plots PC and P2–P4 were taken at a depth of 0–20 cm and prepared for analysis according to the method described elsewhere (Pidlisnyuk *et al.*, 2022). Briefly, the soil samples were dried at 105 °C to constant weight, cleaned of small stones, plant parts and other debris; larger soil clods were ground using a porcelain mortar and pestle. Subsequently, the average soil samples were prepared, passed through a 0.25 mm sieve and used for analysis of metal concentrations.

Samples of plant aboveground organs (stems and leaves) were prepared according to the standard method (DSTU ISO 11465-2001). Plant material was dried at a temperature of 105 °C to constant weight, cooled in desiccators for 1 hour and weighed. The dried samples were burned at 400 °C for 4 hours, cooled in desiccators for 1 hour, weighed and used for analysis (Pidlisnyuk *et al.*, 2022).

In the samples of soil and plant material, the concentrations of metals such as calcium (Ca), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) were determined. The evaluation of metal contents in the soil and plant samples was carried out using X-ray fluorescence analysis following the United States Environmental Protection Agency standard (USEPA, 2007) using an Elvax Light SDD Analyzer, Elvatech, Kyiv, Ukraine. Samples of soil and plant organs for metal concentration analysis were collected in triplicate in each experimental variant. The concentrations of metals were expressed in mg per 1 kg dry weight (DW).

Bioaccumulation factors (BFs) of metals were calculated using the equation: $BF = C_{plant}/C_{soil}$, where C_{plant} is the concentration of an individual element in plant organs (mg kg^{-1}), and C_{soil} is the content of the same element in the soil (mg kg^{-1}) (Mirecki *et al.*, 2015).

Analysis of photosynthetic pigment concentrations. To analyze the concentrations of photosynthetic pigments, the plant leaves were prepared using routine methods. Pieces of leaves were cut from the central parts of fresh leaf blades, crushed and extracted with 96 % ethanol under dark conditions. The mixture was filtered, and the concentration of chlorophyll *a*- and *b*-types (Chl_a and Chl_b, respectively), total chlorophyll concentration (Chl_{a+b}) and content of carotenoids were determined (Lichtenthaler & Wellburn, 1983). The absorbance of the pigment solution was measured using a ULAB-102 spectrophotometer (China) at 470, 649 and 665 nm. The final concentrations of pigments were calculated taking into account the weight of the portion of plant material taken for analysis and expressed in milligrams per 1 g of fresh tissue weight (FW). Plant material was taken for analysis in five replicates.

Processing of the results. The results were processed using variation statistics methods (Welham *et al.*, 2015). Statistical analysis of the results was performed using Excel software. During statistical analysis of the data, the arithmetic mean and standard deviation (M ± S.D.) were calculated. When comparing data groups, the significance of differences was assessed using Student's *t*-test, and the Bonferroni correction was applied for multiple comparisons (Andrade, 2019). After Bonferroni correction, differences between data groups were considered significant at $p < 0.025$.

RESULTS AND DISCUSSION

Effects of oil contamination and humic preparations on plant morphological parameters and photosynthetic pigment concentrations. Available research data show that petroleum hydrocarbons, especially at high concentrations, can disrupt metabolic activity and negatively affect plant development (Athar *et al.*, 2016; da Silva Correa *et al.*, 2022; Roy *et al.*, 2023). However, the results of our study indicate that crude oil contamination of soil does not affect the growth performance of rhizomatous *M. giganteus* plants. Namely, the values of parameters such as shoot height and leaf width in plants from plot P2 were close to those in plants grown on the control plot (**Table 2**), indicating the high adaptability of *M. giganteus* to oil-contaminated soil. Treatment of plants with the Fulvital humic preparation further improved the growth of *M. giganteus*, apparently increasing plant adaptability to oil-contaminated soil. In particular, the shoot height of plants from plot P3 markedly increased compared to this parameter in plants from both plots P1 and P2 ($p < 0.001$). Instead, treatment with the Humifield preparation (plot P4) did not influence the growth parameters of plants cultivated under conditions of soil contamination.

Table 2. **Morphological parameters of *M. giganteus* plants cultivated on oil-contaminated soil and treated with humic preparations (M ± S.D, n = 5)**

Plot number	Shoot height, cm	Leaf width, cm
P1	86.5±5.1	1.5±0.1
P2	84.0±2.2	1.4±0.1
P3	108.3±5.1[a,b]	1.7±0.2[c]
P4	82.5±4.2	1.4±0.1

Note: [a] – significant difference compared to plants from plot P1 ($p < 0.001$); [b] – significant difference compared to plants from plot P2 ($p < 0.001$); [c] – significant difference compared to plants from plot P2 ($p < 0.025$)

One of the most important parameters of plant viability is the photosynthetic system, the efficiency of which significantly affects the plant growth, biomass accumulation and productivity (Terek, 2007; Stirbet *et al.*, 2020). The functioning of the photosynthetic apparatus is highly sensitive to environmental fluctuations and pollution (Polishchuk & Antonyak, 2022; Li *et al.*, 2024). To assess the effect of oil pollution and growth regulators on the photosynthetic system in *M. giganteus*, the concentrations of pigments (chlorophylls and carotenoids) in the leaves of plants were studied under conditions of growth on oil-contaminated soil and treatment with humic preparations.

According to the obtained results, the cultivation of *M. giganteus* plants on oil-contaminated soil resulted in a significant decrease in the concentration of photosynthetic pigments, which was most pronounced at the initial stage of the experiment. In plant samples collected in July from plot P2, the concentrations of Chl_a, Chl_{a+b} and carotenoids were reduced by 1.27–1.30 times ($p < 0.01$) compared to the corresponding values in plant material from plot P1 (**Table 3**). In September, a decrease in the Chl_b concentration ($p < 0.01$) was observed in the leaves of plants from plot P2 compared to that in plants from plot P1.

Table 3. **Concentrations of photosynthetic pigments (mg g^{-1} FW) in *M. giganteus* leaves cultivated on oil-contaminated soil and exposed to treatment with humic preparations (M ± S.D, n = 5)**

Plot	Chl_{a+b}	Chl_a	Chl_b	Carotenoids
P1 (July)	3.24±0.27	2.50±0.24	0.74±0.08	0.88±0.11
P1 (September)	2.08±0.18	1.48±0.12	0.60±0.05	0.43±0.04
P2 (July)	2.55±0.28[a]	1.93±0.21[a]	0.62±0.06	0.68±0.07[a]
P2 (September)	2.01±0.18	1.56±0.17	0.45±0.05[a]	0.50±0.06
P3 (July)	3.77±0.42[b]	2.81±0.30[b]	0.96±0.12[a,b]	0.75±0.09
P3 (September)	1.65±0.19	1.27±0.14	0.38±0.05	0.42±0.05
P4 (July)	2.57±0.28[a]	1.98±0.22[a]	0.59±0.08[a]	0.58±0.06[a]
P4 (September)	2.45±0.22[a,b]	1.94±0.20[a]	0.51±0.04	0.62±0.06[a,b]

Note: [a] – significant difference compared to plants from plot P1 in a specified month ($p < 0.025–0.001$); [b] – significant difference compared to plants from plot P2 in a specified month ($p < 0.025–0.001$)

The decrease in the concentrations of chlorophyll can be attributed to its degradation due to oxidative damage caused by the entry of petroleum hydrocarbons into plants under conditions of soil contamination with crude oil (Moradi *et al.*, 2020). Since chlorophyll is the main photosynthetic pigment of plants and carotenoids play an additional role in photosynthetic reactions (Hashimoto *et al.*, 2016), a decrease in the concentrations of both groups of pigments may lead to the suppression of the photosynthesis process in *M. giganteus* plants grown under oil pollution conditions. The inhibition of photosynthesis was also observed by other authors who studied the effects of petroleum products on various plant species (Mostafa *et al.*, 2021; Hussein *et al.*, 2022).

However, the reduction in the concentration of photosynthetic pigments in plants grown in oil-contaminated soil did not affect the growth performance of *M. giganteus*, as shown in **Table 2**. This effect is of considerable interest since photosynthesis is known to be an important factor in plant productivity (Stirbet *et al.*, 2020). Apparently, the stability of plant growth parameters may be due to the hormetic effect of low concentrations of crude oil on plant growth processes. The absence of an inhibitory effect and even a stimulating influence of low concentrations of petroleum on the growth of a number of plant species was also observed by other authors, associating this phenomenon with a change in the phytohormonal status of plants, an increase in the content of some low-molecular antioxidants, and the presence of plant growth stimulants in crude oil (Adieze *et al.*, 2012; Rodríguez-Rodríguez *et al.*, 2016; Orocio-Carrillo *et al.*, 2024; Abbasov *et al.*, 2024).

Treatment with Fulvital had a pronounced stimulating effect on the chlorophyll content in *M. giganteus* leaves grown on oil-contaminated soil at the initial stage of the experiment. Specifically, Chl_{a+b}, Chl_a and Chl_b concentrations in *M. giganteus* leaves from plot P3 analyzed in July were 1.46–1.55 times higher ($p < 0.001$) than those in plants from oil-contaminated soil of plot P2 (**Table 3**). In addition, treatment with Fulvital led to the normalization of carotenoid level in the leaves of plants grown on oil-contaminated soil of P3 plot.

In contrast, treatment of plants with Humifield had a positive effect on pigment concentrations in the autumn month. In plant leaves taken from plot P4 in September, the concentrations of Chl_{a+b} and carotenoids were increased by 1.22–1.24 times ($p < 0.025$–0.01) compared to those recorded in plants from plot P2 in the same month. Moreover, treatment of plants with this preparation resulted in a significant increase ($p < 0.025$–0.001) in Chl_a, Chl_{a+b} and carotenoid levels compared to the values recorded in leaves of plants from the uncontaminated P1 plot (**Table 3**).

Effects of oil contamination and humic preparations on metal contents in the rhizosphere and aboveground organs of *M. giganteus*. Crude oil is known to contain certain amounts of heavy metals, which may have additional adverse effects on plants and increase metal pollution in the soil (Chinedu & Chukwuemeka, 2018; Gan *et al.*, 2022; Wyszkowski & Kordala, 2022; Aradhi *et al.*, 2023). Currently, high concentrations of heavy metals in the pedosphere and other environmental components have become a global problem due to their destructive impact on various groups of biota and human health (Snitynskyi *et al.*, 1999; Polishchuk & Antonyak, 2019; Rahman & Singh, 2020). On the other hand, oil pollution significantly modifies soil characteristics, including pH and water-air conditions, which affects the mobility and bioavailability of macronutrients necessary for plant nutrition (Dzura *et al.*, 2007; Athar *et al.*, 2016). However, plants growing on contaminated soil can influence its elemental composition due to the absorption capacity of roots and the secretion of organic acids, which affect the mobility of metals in the rhizosphere (Chen *et al.*, 2017). Associated rhizosphere microorganisms can additionally influence the dynamics of metals in the soil adjacent to the root system (Agarwal *et al.*, 2024). Considering these facts, we analyzed the accumulation of some metals in the rhizosphere and aboveground organs of *M. giganteus* cultivated on oil-polluted soil, and the influence of humic preparations on this process.

According to the obtained results, the cultivation of *M. giganteus* plants on the oil-contaminated P2 plot did not cause statistically significant changes in the content of most of the studied metals in the rhizosphere soil (**Table 4**). The exceptions were the concentrations of Cr and Ni, which were more than 1.5 times higher ($p < 0.025$) in the soil of P2 plot than in the soil of the PC plot used as a control. This effect is apparently due to the presence of certain amounts of these and other trace metals in crude oil (Chinedu & Chukwuemeka, 2018; Gan *et al.*, 2022). Other authors have also noted an elevated content of several heavy metals in soils contaminated with oil and petroleum products (Wyszkowski & Kordala, 2022; Aradhi *et al.*, 2023).

Table 4. **Concentration of metals in the rhizosphere soil of plants growing on plots exposed to various experimental conditions (M ± S.D., n = 3)**

Tested metals	Experimental plots			
	PC	P2	P3	P4
Trace metals, mg kg^{-1}				
Cr	88.08±16.21	136.6±13.2[a]	123.6±22.0	111.3±21.8
Cu	16.25±2.72	16.27±3.48	16.75±3.12	17.57±3.78
Mn	600.3±53.8	750.2±72.6	851.4±87.2[a]	703.8±61.4
Ni	14.25±2.10	21.86±2.91[a]	31.43±5.15[a]	20.72±3.44
Pb	23.87±2.24	29.97±3.12	26.69±3.91	27.57±4.03
Zn	52.66±5.75	68.50±7.42	63.20±6.56	66.15±6.04
Other metals, g kg^{-1}				
Ca	3.04±0.52	4.29±0.70	5.18±1.00[a]	3.49±0.78
Fe	22.59±2.40	27.69±3.12	24.89±2.78	25.75±2.65
K	20.26±2.31	22.91±1.97	22.60±2.40	23.28±1.88
Mg	9.20±0.90	7.28±0.74	8.21±0.86	9.89±0.93

Note: [a] – significant difference compared to plants from plot PC ($p < 0.025$–0.01)

Growing *M. giganteus* plants treated with Fulvital increased the content of Mn, Ni and Ca in the oil-contaminated soil of the P3 plot by 1.42, 2.21 and 1.70 times ($p < 0.025$–0.01), respectively, compared to the content of these metals in the soil of the PC plot (**Table 4**).

It should be noted that among the trace metals analyzed in this work, Cu, Fe, Mn, Ni and Zn are essential micronutrients for plants, required in certain quantities for plant growth and development (Kabata-Pendias & Pendias, 2001). However, the presence of elevated levels of trace elements in environmental components and their absorption in increased amounts can have detrimental effects on plant metabolism and physiology (Kabata-Pendias & Pendias, 2001; Ali & Gill, 2022). The uptake of metals (both macronutrients and trace elements) by plants is known to depend on the chemical and biological conditions in the rhizosphere, as well as on processes that can influence the distribution of metals in the soil and their bioavailability (Senila & Kovacs, 2024). Consequently, the assessment of metal concentrations in *M. giganteus* plant tissues and calculation of bioaccumulation factors (BFs) can provide information on soil-plant interactions and on the selectivity of plant compartments for different elements.

The results of this study reveal contrasting differences in metal accumulation levels between leaves and stems of *M. giganteus* plants cultivated on oil-contaminated soil (**Table 5**). In contrast to the stability of metal concentrations in plant leaves, the stems of plants from plot P2 had a significantly reduced content of all analyzed metals compared to plants from plot P1 (Table 5). Namely, the levels of such macronutrients as Ca, K and Mg in stems were reduced by 4.29, 3.37 and 6.16 times ($p < 0.001$), respectively, and the concentrations of Cr, Fe, Ni and Zn were 1.31–2.79 times lower ($p < 0.025$–0.001) in plants from the P2 plot than those recorded in plants from the P1 plot. These results are consistent with previously obtained data on the decrease in the content of trace elements in the shoots of *Carex hirta* L. species grown on oil-contaminated soil (Dzura *et al.*, 2007).

Table 5. **Metal concentrations (mg kg^{-1}) in aboveground organs of *M. giganteus* plants grown under different experimental conditions (M ± S.D., n = 3)**

Tested metals	Experimental plots			
	P1	P2	P3	P4
Leaf				
Ca	12947±995	10555±820	11513±891	9851±922
Cr	13.07±1.47	12.89±1.38	10.47±1.30	10.11±1.43
Fe	741.1±65.8	775.4±89.1	687.0±44.7	724.9±76.3
K	6503±618	6784±562	7200±612	7350±547
Mg	949.4±76.2	1102±84	1231±97^{a}	984.3±99,0
Ni	115.6±9.1	98.36±7.68	124.0±11.5	130.8±14.1^{b}
Zn	5.17±0.48	4.74±0.31	5.22±0.58	4.88±0.43
Stem				
Ca	4311±470	1004±137^{a}	1260±184^{a}	1647±228^{a,b}
Cr	5.25±0.60	2.19±0.28^{a}	3.42±0.52^{a,b}	2.51±0.31^{a}
Fe	493.4±52.2	176.9±20.5^{a}	191.2±22.7^{a}	266.2±28.4^{a,b}
K	4162±427	1236±213^{a}	2028±279^{a,b}	2997±375^{a,b}
Mg	499.6±57.4	81.11±8.73^{a}	110.1±12.0^{a}	227.2±23.9^{a,b}
Ni	12.62±1.10	9.62±0.98^{a}	13.23±1.18^{b}	8.30±0.94^{a}
Zn	2.40±0.20	0.96±0.09^{a}	1.30±0.14^{a,b}	1.93±0.17^{b}

Note: ^{a} – significant difference compared to plants from plot P1 ($p < 0.025$–0.001); ^{b} – significant difference compared to plants from plot P2 ($p < 0.025$–0.001)

According to the obtained results, treatment with Fulvital and Humifield resulted in elevated concentrations of Mg and Ni ($p < 0.025$), in the leaves of plants from plots P3 and P4, respectively, compared to those in plants from plot P1 (**Table 5**). Magnesium is known to be essential for vital plant functions; primarily, Mg^{2+} is a central component of the chlorophyll molecule and supports the function of absorbing sunlight during photosynthesis (Tian *et al.*, 2021). Therefore, the improvement of Mg supply to *M. giganteus* leaves under the influence of Fulvital treatment may have a positive effect on chlorophyll synthesis and the process of photosynthesis in plant cells. This effect is especially important in connection with the above-mentioned decrease in chlorophyll content in the leaves of *M. giganteus* plants grown under oil pollution conditions (**Table 3**).

Treatment of plants with Fulvital (plot P3) caused an increase in the concentration of Cr, K, Ni and Zn ($p < 0.025$), whereas treatment with Humifield (plot P4) led to a significant rise ($p < 0.025$–0.001) in the content of macronutrients (Ca, K and Mg) and micronutrients (Fe and Zn) in plant stems compared to those in plants from plot P2 (**Table 5**). However, concentrations of most metals determined in plant stems from plots P3 and P4 remained below the levels recorded in the stems of plants from plot P1.

The results of calculating the bioaccumulation factors demonstrate that the BF values of studied metals in the leaves of *M. giganteus* grown on plot P1 can be arranged in descending order as follows: Ni > Ca > K > Cr > Mg > Zn > Fe, whereas in the plant stem, the BF values of the metals are arranged as follows: Ca > Ni >K > Cr > Mg > Zn > Fe (**Table 6**). Overall, *M. giganteus* leaves showed higher BF values and bioaccumulation levels of all tested metals than plant stems.

Table 6. **Bioaccumulation factors of metals in aboveground organs of *M. giganteus* plants**

Metals	P1		P2		P3		P4	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Ca	4.25	1.416	2.46	0.234	2.22	0.243	0.091	0.023
Cr	0.148	0.060	0.094	0.016	0.085	0.028	0.091	0.023
Fe	0.033	0.022	0.028	0.006	0.028	0.008	0.028	0.010
K	0.321	0.205	0.296	0.054	0.319	0.090	0.316	0.129
Mg	0.103	0.054	0.151	0.011	0.150	0.013	0.100	0.023
Ni	8.11	0.89	4.50	0.44	3.95	0.42	6.31	0.40
Zn	0.098	0.046	0.069	0.014	0.083	0.021	0.074	0.029

BF values for most metals tended to decline in both leaves and stems of *M. giganteus* grown on oil-contaminated soils, except for BF value for Mg, which showed an increasing trend in leaves of plants from the oil-polluted P2 plot (**Table 6**). Treatment of plants with humic preparations resulted in a slight increase in BF values for several metals such as K, Ni and Zn in the aboveground organs of *M. giganteus* grown in plots P3 and P4, as compared to those in plants from plot P2.

It was characteristic that the leaves of *M. giganteus* from all plots had the values BF >1 for Ni, which demonstrated the high capacity of plants to bioaccumulate this metal by extracting it from both contaminated and uncontaminated soils. Nickel is an essential micronutrient for plants, but it can also have harmful effects on plant metabolism, depending on the concentration (Hassan *et al.*, 2019). The element is easily bioavailable to plants, especially in acidic soils. In this study, the level of Ni accumulation in *M. giganteus* was found to be higher than that in other plant species (Kabata-Pendias & Pendias, 2001). However, we did not observe any external signs of metal toxicity in *M. giganteus* plants. These results may indicate the ability of *M. giganteus* plants to tolerate elevated Ni concentrations when extracting this metal from the soil.

The BF >1 values were also recorded for Ca in leaves of plants from plots P1–P3 and in plant stems from plot P1. Calcium cation (Ca^{2+}) is known to be an essential nutrient for plant growth and also an important signaling component acting as a second messenger in the transduction of hormonal signals, particularly abscisic acid signals (Batistič & Kudla, 2012). Activation of Ca^{2+}-mediated signaling pathway improves plant adaptation to environmental stresses. It can be assumed that the potentiality of *M. giganteus* to extract this element from the soil may have a positive effect both on meeting its calcium needs and on the plant's ability to respond to adverse environmental conditions.

The leaves of *M. giganteus* from all plots showed a medium accumulation potential for Mg and K (BF values ranged from 0.1 to 0.321); medium accumulation was also observed for Cr in the leaves of plants from uncontaminated soil on plot P1. At the same time, the accumulation potential for Cr in leaves from oil-contaminated plots P2–P4, as well as for Fe and Zn in leaves from all experimental plots, was low (BF <0.1), indicating a restricted transport of these elements into the aboveground parts of *M. giganteus* plants. The lowest values of bioaccumulation factor (BF ≤0.01) were found for Fe in the stems of *M. giganteus* from plots P2–P4, indicating a lack of plant ability to accumulate Fe from oil-contaminated soil, regardless of the treatment of plants with humic preparations.

The obtained results indicate that the cultivation of *M. giganteus* on oil-contaminated soil does not lead to an increased accumulation of the studied metals in its aboveground organs compared to plants grown on uncontaminated soil. Our results are consistent with those obtained by F. Nsanganwimana *et al.* (2021), who reported accumulation of heavy metals in the roots of *M. giganteus* and low metal transfer rates to the leaves and stems of the plant. At the same time, the high BF value for Ni found in the leaves and stems of *M. giganteus* indicates the potentiality of plants to extract this metal from contaminated soils, which may contribute to the effectiveness of soil phytoremediation using *M. giganteus* plants.

CONCLUSIONS

The results of the study suggest that *M. giganteus* plants exhibit good adaptability to cultivation on oil-contaminated podzol soil, as evidenced by the stability of morphological parameters in plants grown on the plot polluted with crude oil. However, the concentrations of chlorophyll and carotenoids in plant leaves decrease under conditions of oil pollution. Therefore, the use of humic preparations that increase the content of photosynthetic pigments in leaves of *M. giganteus* obviously has a beneficial effect on plant growth and enhances plant resistance to oil contamination. Humic substances also influence the metal concentrations in the rhizosphere soil and contribute to an increase in the level of macronutrients and some trace elements in aerial parts (mainly in the stem) of *M. giganteus* compared to those in plants grown on an oil-contaminated plot untreated with these substances. Overall, this study did not reveal elevated metal accumulation in the aboveground organs of *M. giganteus*, which could have a positive impact on the quality of plant biomass used for biofuel production. Considering the obtained results, it can be concluded that the cultivation of biofuel crop *M. giganteus*, especially in combination with its treatment with humic preparations, will be promising on podzol soils contaminated with oil and petroleum products due to a high adaptability of the plant to oil pollution and a good quality of the harvested biomass. Furthermore, the potential of the plant to extract Ni from soil, as evidenced by the high bioaccumulation factor for this metal, may mediate the ability of *M. giganteus* to clean-up contaminated soils.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal Rights: this article does not contain any studies with animal subjects performed by any of the authors.

Human Rights: this article does not contain any studies with human subjects performed by any of the authors.

AUTHOR CONTRIBUTIONS

Conceptualization, [N.D.; H.A.]; methodology, [N.D.; P.S.]; validation, [N.D.; H.A.]; formal analysis, [N.D.; I.P.]; investigation, [N.D.; I.P., P.S.; O.R.]; resources, [N.D.; P.S.]; data curation, [N.D.; P.S.; H.A.]; writing – original draft preparation, [N.D.; I.P.; H.A.]; writing – review and editing, [N.D.; H.A.]; visualization, [I.P.] supervision, [N.D.; H.A.]; project administration, [N.D.; H.A.]; funding acquisition, [-].

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ФІЗІОЛОГІЧНІ ПАРАМЕТРИ ТА МЕТАЛОАКУМУЛЯЦІЙНА ЗДАТНІСТЬ БІОЕНЕРГЕТИЧНОЇ РОСЛИНИ *MISCANTHUS × GIGANTEUS* ЗА УМОВ ВИРОЩУВАННЯ НА ЗАБРУДНЕНОМУ НАФТОЮ ПІДЗОЛИСТОМУ ҐРУНТІ Й ОБРОБКИ ГУМІНОВИМИ ПРЕПАРАТАМИ

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Актуальність. Дослідження фізіологічних особливостей *Miscanthus × giganteus* J. M. Greef, Deuter ex Hodk. & Renvoize актуальні з огляду на фіторемедіаційний потенціал цієї біоенергетичної рослини. Метою роботи було дослідити вміст фотосинтетичних пігментів у листках *M. giganteus*, акумуляцію металів у ризосфері та надземних органах, а також морфологічні показники за умов вирощування рослин на забрудненому нафтою ґрунті й обробки гуміновими препаратами.

Матеріали і методи. Під час проведення польових дослідів закладено п’ять дослідних ділянок (ДК та Д1–Д4) площею 1 м^2 на території, прилеглій до Старосамбірського нафтового родовища, на підзолистому ґрунті. Ділянка ДК слугувала як контроль під час досліджень вмісту металів у ґрунті. У ґрунт на ділянці Д1 висаджували ризоми *M. giganteus*; у ґрунт на ділянках Д2–Д4 вносили 10 л/м^2 сирої нафти, а потім висаджували ризоми *M. giganteus*. Перед висаджуванням на ділянках Д3 і Д4 ризоми замочували в розчинах гумінових препаратів (відповідно, Fulvital® Plus Liquid і Humifield® Forte). У період росту рослини двічі обприскували гуміновими препаратами.

Висоту пагона, ширину листка, концентрацію хлорофілу *a*- та *b*-типу (відповідно, Chl_a та Chl_b), загальний вміст хлорофілу (Chl_{a+b}) та концентрацію каротиноїдів аналізували стандартними методами. Вміст металів (Ca, Cr, Cu, Fe, K, Mg, Mn, Ni, Pb, Zn) у зразках ґрунту й органах рослин визначали методом рентгенофлуоресцентного аналізу за допомогою аналізатора Elvax Light SDD.

Результати. Вирощування на забрудненому нафтою ґрунті не впливало на висоту пагонів і ширину листків *M. giganteus*, але знижувало вміст Chl_a, Chl_b, Chl_{a+b} і каротиноїдів у листках рослин. Обробка рослин гуміновими препаратами призводила до підвищення концентрації пігментів у листках у різні періоди росту. В забрудненому нафтою ґрунті, засадженому *M. giganteus*, виявлено підвищення концентрації Cr і Ni. За обробки *M. giganteus* препаратом Fulvital® Plus Liquid виявлено збільшення вмісту Ca, Mn та Ni у ризосферному ґрунті на забруднених нафтою ділянках. Вирощування *M. giganteus* на забрудненому нафтою ґрунті спричинило зниження концентрації Ca, Cr, Fe, K, Mg, Ni та Zn у стеблі рослин. Обробка гуміновими препаратами підвищувала вміст зазначених металів у стеблі та концентрацію Mg і Ni в листках рослин, вирощуваних на забрудненому нафтою ґрунті. Відповідно до значення коефіцієнтів біоакумуляції (КБ) металів, листки *M. giganteus* характеризуються високим потенціалом накопичення Ni та Ca (КБ >1), помірним потенціалом накопичення Mg, K і Cr (КБ у межах 0,1–0,32) і низьким потенціалом накопичення Fe і Zn (КБ <0,1). Вирощування на забрудненому нафтою ґрунті призводило до зниження значень КБ металів у листках і стеблі рослин.

Висновки. Обробка гуміновими препаратами позитивно впливає на фізіологічні показники *M. giganteus* за вирощування на забрудненому нафтою підзолистому ґрунті. Здатність *M. giganteus* до фітоекстракції Ni з ґрунту може опосередковувати фіторемедіаційний потенціал рослини. У зв’язку з цим вирощування *M. giganteus* у поєднанні з обробкою рослин гуміновими препаратами перспективне на ґрунтах, забруднених нафтою та нафтопродуктами.

Ключові слова: *Miscanthus* × *giganteus*, біоенергетичні рослини, забруднений нафтою ґрунт, підзолисті ґрунти, гумінові препарати, фіторемедіація, важкі метали, фотосинтез

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