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Original Research

Accumulation of Heavy Metals in Plant Biomass from the Mixed Culture of *Zea Mays* and *Phaseolus Vulgaris*, and Potential Biomass Use for Methane Production

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Abstract

Contemporary agriculture has to face many challenges that are connected with climate change but also with the intensive form of farming. Soil erosion, loss of biodiversity, droughts, soil degradation and pollution with heavy metals (HM) are only a few of the problems. In the presented study, the term "heavy metals" is used operationally to denote metallic elements (Cr, Cd, Cu, Ni, Hg, Zn and Fe), investigated in relation to their accumulation in plant biomass and their potential effects on the process of anaerobic digestion. The use of mixed cropping systems can be an efficient tool to reduce some of these negative aspects. In this study, a possible use of the mixed cropping system (MCS) including *Zea mays* and *Phaseolus vulgaris* for phytoremediation processes and methane production was assessed in a two-year experiment. The crops were grown on fields with naturally abundant HM. The HM were accumulated more in legumes than in maize variants. There were no significant differences between HM concentrations in the pure maize culture (M) and maize grown in the MCS. The highest biological accumulation coefficient (BAC) was observed in Cd (1.06) in Variant M in 2021. The calculated theoretical methane yield (TMY) per field growth area (m^3/ha) indicated that using the MCS composed of maize and bean led to a higher theoretical methane yield. TMY of the MCS was higher than TMY of the pure maize culture (M) on average by 9.61% in 2020 and by 28.07% in 2021.

Keywords: biogas plant, legumes, phytoextraction, heavy metals, metal accumulation, phytoremediation

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Introduction

Modern agriculture currently faces many challenges such as soil erosion [1, 2], anthropogenic pollution by xenobiotics [3-5] and abiotic and biotic stresses [6, 7]. These challenges are accompanied by increasing food demands caused by the quickly growing global population, and by the demand for greener energy [8]. Therefore, effective environment-friendly cropping systems should be taken into account. Apart from agricultural monocultures, mixed cropping systems or mixed cultures (MCS) meet many of these requirements [9].

MCS are generally known as agricultural systems composed of more than one crop on one site at the same time [10]. The most often-used intercrops belong to the Poaceae and Fabaceae families [11, 12]. The use of legumes in these systems has been intensively studied [13-16] due to their positive effects on soil conditions [17] and their ability to reduce interspecific competition through biological nitrogen fixation [14]. It was shown that the transport of N from N-fixing legumes to non-fixing species takes place through the decomposition of N-fixing plants, N exudation and mycorrhizal symbiosis [18]. Research showed that MCS provide interesting results on the large scales of observed parameters, and that they are often more advantageous than crop monocultures [19].

Demand for renewable energy sources is currently increasing, which results in an increasing number of crops grown for the purpose. Due to high yields and highly innovative crop breeding techniques, biogas is currently produced mainly from the pure maize crops grown on large fields [10, 20]. This often leads to competition between the crops grown for biogas production and the crops grown for food supply, because the available agricultural soil is limited. According to Soha et al. [21], MCS can mitigate this negative aspect because they represent an effective solution for using farmland.

However, the production of methane in biogas plants can be affected by the presence of various xenobiotics such as HM in biomass. In general, sources of HM in the environment are natural and anthropogenic. In recent years, considerable attention has been paid to the issue of HM contents in the soil, as reported by Wang et al. [22]. HM can enter the plant biomass thanks to the phytoremediation ability of plants and thus they can subsequently contaminate biogas plants. There are various studies assessing the ability of plants to accumulate metals and their potential use in phytoremediation [23]. On the other hand, it should be noted that the presence of many HM is essential for the growth of plants and for microorganisms involved in the anaerobic degradation of biomass, since they are crucial for the proper functioning of various enzymes [24]. Nevertheless, the increasing concentration of HM in the plant biomass can negatively influence the methane yield [25]. Some studies suggest that biomass produced in MCS with legumes as cover crops contains a higher concentration of Cd when compared to the biomass from the monoculture. Cowpea, soybean and Japanese clover significantly increased the content of Cd in *Zea mays* [26]. According to Zhang et al. [27], cumulative yields of biogas amounted in rape, oat and wheat respectively to 159.37%, 179.23% and 111.34% as compared with the control when the Cd content in the above-ground biomass of these three crops was 2.00±0.44, 39.80±1.25 and 6.37±0.15 mg/kg of biomass, respectively. On the other hand, high Cd concentrations (> 20 mg/L) inhibited acidogenesis [27, 28].

No effects of trace elements in the plants on bioenergy production were observed in the case of *Helianthus annuus* and *Silybum marianum* grown on the mining soil contaminated with trace elements [29]. Similarly, anaerobic digestion of plant residues obtained from *Phragmites australis, Arundo donax* and *Piptatherum miliaceum* was shown to be a suitable final destination of plant biomass obtained through phytoremediation processes [30]. In this study, the suitability of the mixed cropping system composed of maize (*Zea mays*) and bean (*Phaseolus vulgaris*) for methane production in a biogas plant was determined by the calculation of theoretical methane yield (TMY), which is often used for the prediction of methane yield produced from the biomass of MCS [16].

The use of MCS combining maize and common bean offers many advantages. It reduces the occurrence of diseases and pests, effectively utilizes nutrients accessible to plants, and suppresses the activity of weeds [12, 31-34]. This confirms that the use of maize and common bean in the MCS leads to higher yields, which points to high utilization of resources.

The aim of this study was to evaluate the effect of a mixed cropping system (maize-bean) on biomass production and its potential use for methane production as compared with the maize monoculture. In addition, the study assessed the accumulation of selected HM in plant biomass as an important factor influencing biomass quality and its suitability for anaerobic digestion. The study tested the following hypotheses (null and alternative): H_0 = Cultivation of maize and bean in the MCS has no impact on their capability to accumulate HM in their biomass; H_1 = MCS alters biomass yield relative to monoculture; H_2 = MCS affects theoretical methane yield (TMY); and H_3 = the cultivation system influences the accumulation of HM in plant biomass.

Materials and Methods

Pedological, Geological and Meteorological Conditions of the Experimental Site

In 2020 and 2021, a 2-year experiment was conducted in the area near Troubsko, Czech Republic (49°17’49.822’’N, 16°49’13.044’’E), which is located in a typical potato-growing region (Fig. 1). In terms of

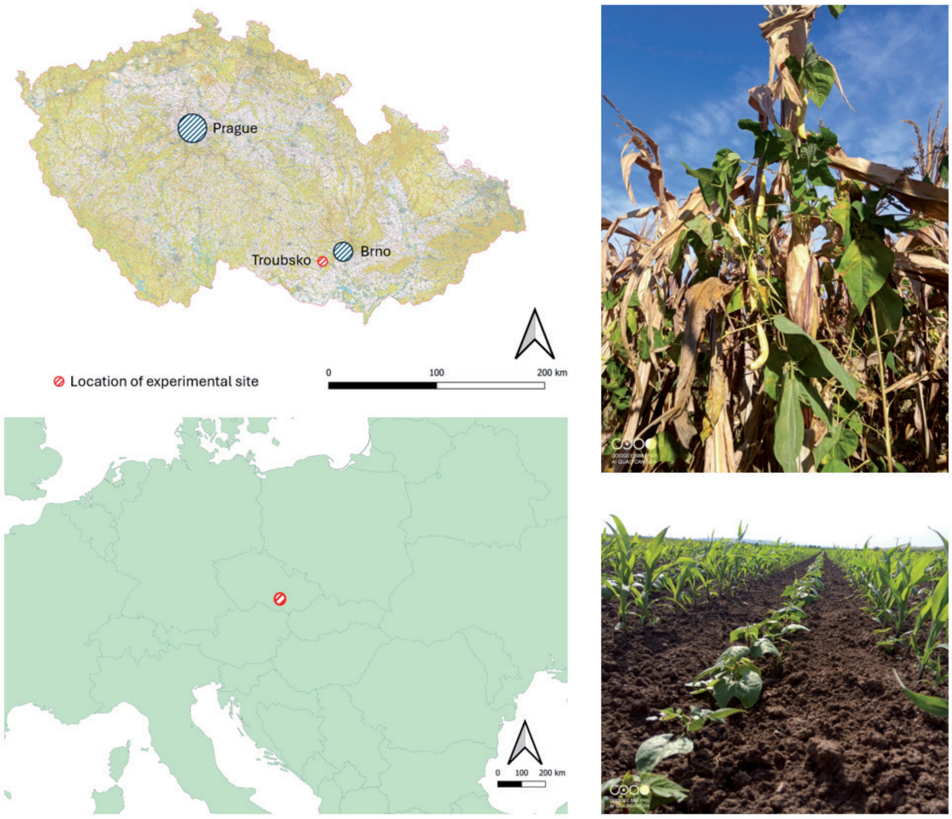

Fig. 1. Location of experiments in Troubsko, Czech Republic.

climatology, it is a moderately warm and moderately dry area with a mean annual temperature of 8.95°C and mean annual precipitation of 525.6 mm. In geological terms, the area is characterized by loess and loess loam of the Bohemian Massif, and the soil type is modal brown earth.

As for meteorological conditions, the year 2021 was wetter (627.8 mm) than the year 2020 (529.4 mm). Precipitation amounts in both years were higher than normal (1981-2010; Table 1). Contrary to precipitation, the temperature in 2021 was lower (8.61ºC) than that in 2020 (10.7ºC). As compared with the normal temperature (1981-2010), the year 2020 was warmer (Table 1) and the year 2021 was colder (Table 2).

Field Experiment Establishment and Collection of Plant and Soil Samples

Three cropping variants were established in the field experiment: (i) maize monoculture (M), where maize was cultivated as a sole crop; (ii) maize grown in a mixed cropping system (M-MCS), where maize

Table 1. Precipitation amounts (mm) and temperatures (ºC) in 2020 on the experimental site in Troubsko and mean precipitation amounts and temperatures between 1981 and 2010.

Year	Precipitation / Month (mm)												Total (mm)	
	1	2	3	4	5	6	7	8	9	10	11	12	Annual	In veg. period
2020	8.5	27.1	25.7	20.3	65.4	87.2	59.0	105.9	81.6	130.1	23.4	36.8	529.4	349.3
1981-2010	25.0	23.9	31.5	32.0	60.5	68.7	71.6	63.7	48.2	32.1	36.4	32.0	525.6	344.7
Year	Temperature / Month (ºC)												Mean (ºC)	
	1	2	3	4	5	6	7	8	9	10	11	12	Annual	In veg. period
2020	-0.2	4.6	5.3	9.9	12.6	18.0	19.2	20.6	15.0	10.1	4.5	2.4	10.7	16.83
1981-2010	-1.7	-0.3	3.8	9.5	14.6	17.4	19.5	18.8	14.1	8.8	3.5	-0.6	8.95	15.65

Note: Weather conditions were measured using a professional meteorological station (rain gauge: MR3H-FC, METEOSERVIS Ltd., CZE; wind gauge: WAA151, VAISALA Ltd., FIN; temperature and humidity sensor: HMP35D, VAISALA Ltd., FIN) operated by the Czech Hydrometeorological Institute in line with international norms for monitoring meteorological parameters [35].

Table 2. Precipitation amounts (mm) and temperatures (ºC) in 2021 on the experimental site in Troubsko and mean precipitation amounts and temperatures between 1981 and 2010.

Year	Precipitation / Month (mm)												Total (mm)	
	1	2	3	4	5	6	7	8	9	10	11	12	Annual	In veg. period
2021	31.9	38.0	21.7	49.3	96.1	73.6	72.7	128.6	23.7	12.6	40.4	39.2	627.8	444.0
1981-2010	25.0	23.9	31.5	32.0	60.5	68.7	71.6	63.7	48.2	32.1	36.4	32.0	525.6	344.7
Year	Temperature / Month (ºC)												Mean (mm)	
	1	2	3	4	5	6	7	8	9	10	11	12	Annual	In veg. period
2021	-0.6	-1.4	3.1	6.1	11.6	18.7	20.0	16.9	14.1	9.0	4.7	1.2	8.61	14.56
1981-2010	-1.7	-0.3	3.8	9.5	14.6	17.4	19.5	18.8	14.1	8.8	3.5	-0.6	8.95	15.65

Note: Weather conditions were measured using a professional meteorological station (rain gauge: MR3H-FC, METEOSERVIS Ltd., CZE; wind gauge: WAA151, VAISALA Ltd., FIN; temperature and humidity sensor: HMP35D, VAISALA Ltd., FIN) operated by the Czech Hydrometeorological Institute in line with international norms for monitoring meteorological parameters [35].

was cultivated together with common bean; and (iii) bean grown in the mixed cropping system (B-MCS), representing bean plants intercropped with maize within the same plots. The B-MCS variant does not represent a pure bean stand but refers exclusively to bean biomass collected from the mixed cropping system.

In both years, the stands of MCS and pure maize (mono) culture were established in the second decade of April within a single soil block using the Kinze 3500 (Kinze Manufacturing, Williamsburg, IA, USA) "interplant system" seeding machine in a single operation. Maize was sown in an amount of 80,000 individuals per hectare. It was grown alone as a pure crop (Variant M) or in MCS (Variant MCS): M-MCS (maize grown in the MCS) with bean (B-MCS) in a ratio of 1:1 (80,000 to 80,000 individuals). Spacing between the M-MCS and B-MCS rows was 37.5 cm (Fig. 2). The crops were treated with BASAGRAN (BASF, Ltd.; 2.1 L/ha with the active substance being bentazon 480 g/l; i.e. 40.3% w.) herbicide only.

Plant and soil samples were collected in each year before the end of the experiment from individual sampling points (Fig. 3). Plant samples collected from each sampling point represented 10 plants. In the case of soil samples, disturbed mixed soil samples were collected from the determined sampling points in three repetitions from each experimental variant (M and MCS) (Fig. 3) from a depth of 0.0-0.3 m.

The yield of produced biomass was determined by direct sampling of plants from the respective experimental variants (Fig. 3) according to the methodology published by Loučka et al. [37]. In wide-row plants, the above methods had to be used as they take into account the spacing between individual rows, the number of plants in the row, and the 10 plants harvested at the growth stage BBCH 77 (milk ripeness) – 83 (early wax ripeness). Sampling dates in the respective experimental years were as follows: a) 2020–2 September; b) 2021–14 September. For each variant (M, MCS), they were weighed to estimate fresh

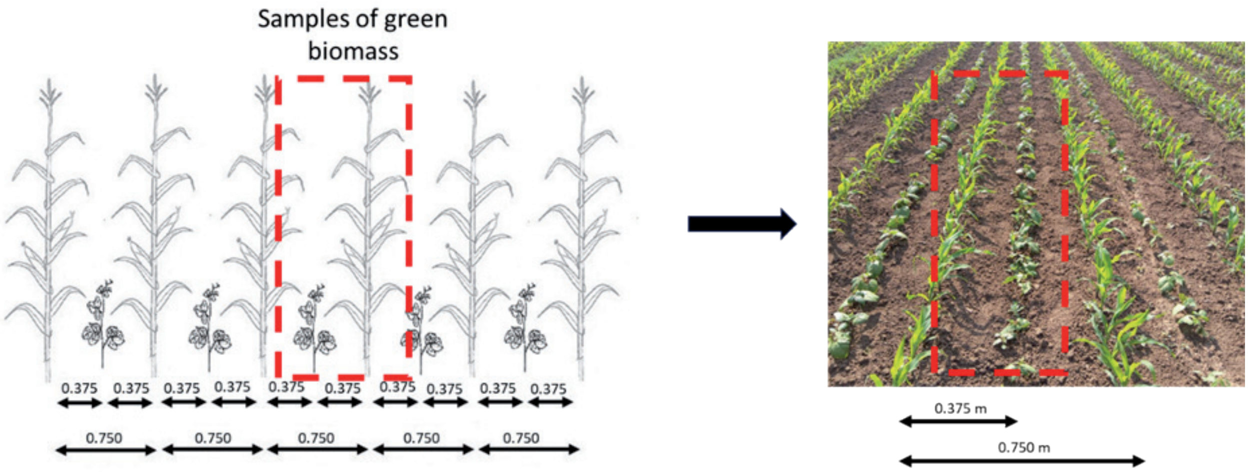

Fig. 2. Scheme of the intercropping system containing maize (M-MCS) and bean (B-MCS) and collection of plant samples according to Kintl et al. [36].

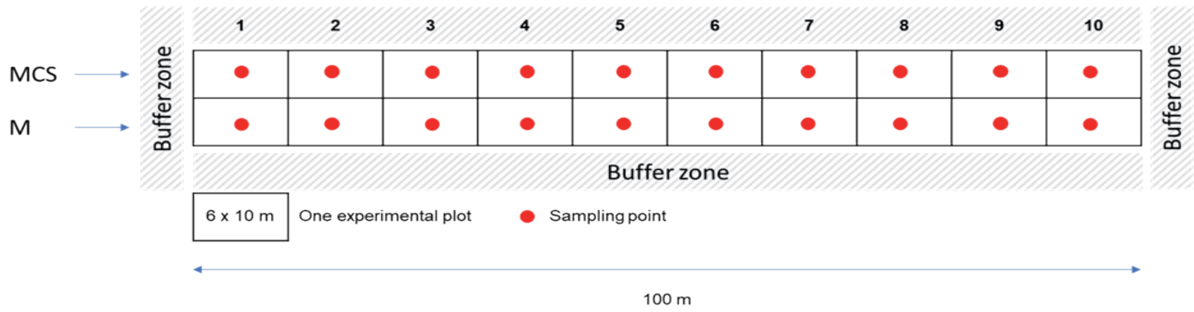

Fig. 3. Organization of the field experiment and the scheme of sampling. One experimental plot included 8 rows of maize and 7 rows of bean in Variant MCS and 8 rows of maize (pure crop) in Variant M. The field experiment was prepared according to the methodology published by Kintl et al. [36].

matter (FM). Subsequently, the plant samples were dried at 60ºC to obtain dry matter (DM) which was also weighed. Yields of FM and DM were calculated according to Loučka et al. [37] (Equation (1)):

$$V = (10/s \cdot p \cdot x \cdot y) \cdot m \tag{1}$$

Where: *V* is the crop yield (t/ha); *m* is the weight of harvested plants (kg); *s* is the row width or mutual distance of rows (m); *p* is the number of rows from which the plants were harvested; *y* is the number of harvested plants and *x* is the spacing of plants in the row (m).

Analysis of Plant and Soil Samples

Samples of soil and biomass from the respective variants – 1. maize grown in the monoculture (M), 2. maize grown in the mixed cropping system (M-MCS) and bean grown in MCS (B-MCS) – were submitted for analysis to a laboratory authorized by the Czech Institute for Accreditation where concentrations of selected HM (Cr, Cd, Cu, Ni, Hg, Zn and Fe) were determined pursuant to ČSN EN ISO 11885 [38]. The content/concentration of HM, defined here as the analysed metallic elements Cr, Cd, Cu, Ni, Hg, Zn and Fe, was determined in the samples to specify a possible influence of growing maize and bean in MCS on their uptake from the soil and presence in the plant biomass.

The plant samples were further analysed for the content of carbohydrates, proteins and lipids in order to calculate the theoretical methane yield (TMY) (Equation (2)). Crude protein was determined using the Kjeldahl method. Protein content was determined using the KjeltecTM 2300 analyser (FOSS, Denmark) and subsequently multiplied by the coefficient of 6.25. To determine the content of lipids, the Soxhlet method was used with water cooling and direct weight extraction by diethyl ether. Crude fibre content was determined by two-step hydrolysis with sulphuric acid and potassium hydroxide, and then, ash content was deducted. During the fibre fraction analysis (ADF, acid detergent fibre; NDF, neutral detergent fibre), acetyltrimethylammonium detergent was used for ADF and lauryl sulphate detergent for NDF [20]. All analyses were made using the ANKOM 220 Fibre Analyser (ANKOM Technology, USA). A detailed description is published in Vitez et al. [20]. Each analysis included a 100 g sample of biomass and was performed in triplicate.

TMY refers to the maximum amount of methane gas that can be produced from a given amount of organic material under ideal conditions. TMY is expressed in terms of volume or mass of methane per unit of organic material. The calculation was made according to Kintl et al. (16), according to Equation (2) [39-42] from values presented in Annex Table A1.

$$TMY = [(0.415 \cdot \%carbohydrates) + (0.496 \cdot \%proteins) + (1.014 \cdot \%lipids)] \;/\; 100\,(m^3/kg_{vs}) \tag{2}$$

Concentrations of HM obtained from the biomass and soil analyses were used to calculate the bioaccumulation factor (BAC) according to Amin et al. [43] (Equation (3)). Bioaccumulation factor (BAC) is a coefficient expressed as a ratio of HM concentration in the shoots (Me_{shoot}) and soil (Me_{soil}), calculated according to the following equation:

$$BAC = Me_{shoot}/Me_{soil} \tag{3}$$

Data Processing

The statistical analysis of acquired data made use of the software STATISTICA 13.5.0.17 (StatSoft, Inc®). Results presented in this study are the means of at least 10 replications for each presented parameter [37], except for the bioaccumulation factor, which is a ratio of the means. Significant differences in the yields of FM and DM and concentrations of HM in the plant biomass were obtained using the analysis of variance following the preceding verification of data normality and homogeneity of variances (ANOVA, P<0.05). In the case of variants where the data assumptions were not fulfilled (Hg, Ni, Fe), the non-parametric test (Kruskal-Wallis) was used. Significant differences among the data were assessed using the post-hoc Tukey's test (FM yield, DM, Cr, Cd, Cu, Zn) or multiple comparisons of mean ranks for all groups (Hg, Zn, Fe) at $P<0.05$.

Results and Discussion

Plant Biomass Production

In the field experiment conducted in 2020-2021, production of biomass by model crops was studied in the form of FM (Fig. 4 and Table 3) and in the form of DM (Fig. 5 and Table 4).

In 2020, FM yields recorded in Variant M and Variant MCS (M-MCS+B-MCS) were 84.99 t/ha and 96.19 t/ha, respectively (sum of maize and bean biomass production in MCS; Fig. 4); the difference between the yields was not significant (Table 3). In contrast, a significant difference was found between the monoculture and the MCS in 2021 (Table 3) when FM yields were 60.36 t/ha in Variant M and 83.08 t/ha in Variant MCS. It means that the difference in yields between the monoculture and the MCS exceeded 22 t/ha (Table 3). As to the comparison of individual years, FM yields in 2021 were 29.0% (Variant M) and 13.6% (Variant MCS) lower than in 2020.

Karasu et al. [44] claim that FM yields of three maize hybrids ranged from 81.5 to 92.9 t/ha, which would correspond to the FM maize values in Variant M and Variant MCS recorded in 2020. On the other hand, a drop in maize yields occurred in 2021 both in Variant M and in Variant MCS in which the decrease was greater.

However, the above-mentioned drop in the yield of maize FM by about 20% in Variant MCS was compensated by the yield of bean FM, and the total biomass production of Variant MCS was thus significantly higher than that of Variant M in 2021. Li et al. [45] conducted experiments with the MCS of four maize and soya varieties in conditions of the Tibetan Plateau. FM yields of pure maize stands ranged from 41.67 to 48.70 t/ha; FM yields of MCS of maize and soya were higher (from 50.48 to 52.59 t/ha) with the density of maize plants being 67,500 per ha and soya plants 135,000 per ha. Thus, a certain decrease of maize yield is assumed in the MCS, which is, however, at least partly compensated by the yield of biomass of another crop grown together with maize. Based on experiments with the MCS of maize and bean, Soe Htet et al. [46] recorded the highest FM yield (46.61 t/ha) in the pure maize stand, and the lowest FM yield was recorded in the pure stand of bean. MCS with the highest FM yield was the variant with the 50:50 maize/bean ratio. Based on the research of Soe Htet et al. [46], it is therefore possible to deduce that the proportion of maize in MCS should not decrease below 50%. This assumption was verified already in some formerly published experiments and studies [15, 16, 47].

The following step consisted in the monitoring of biomass yield in the form of DM. In 2020, Variant M exhibited a yield of 31.86 t/ha DM and Variant MCS (M-MCS+B-MCS) exhibited a yield of 35.18 t/ha (Fig. 5, Table 4). In 2021, the two variants exhibited 26.54 t/ha (M) and 29.00 t/ha (MCS). The course of values and their development in the respective years corresponded with the FM yield values. The results indicate that as compared with 2020, DM yields in 2021 were 17.6% (MCS) and 16.7% (M) lower. A significant difference was observed only between the yields of maize in the monoculture and maize in the MCS in 2021 (Table 4). A likely reason for the year-on-year differences in FM and DM yields was colder and more

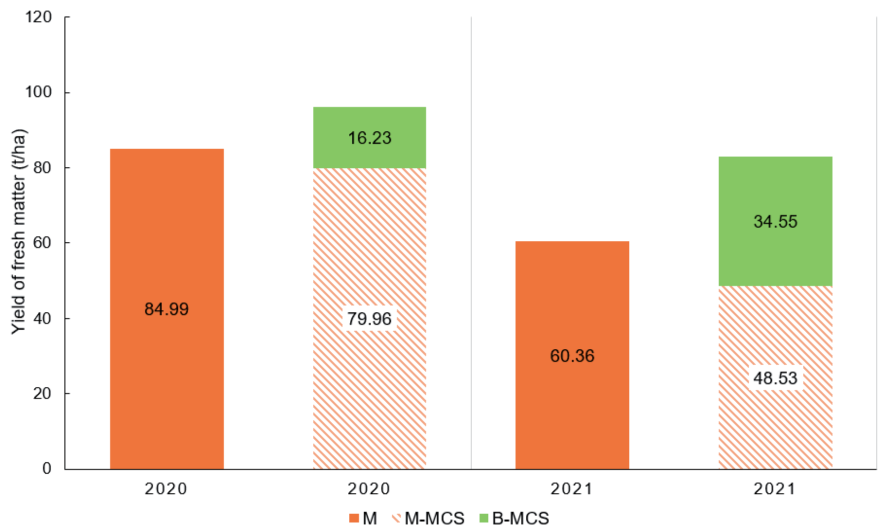

Fig. 4. Yields of fresh matter (FM) in the maize monoculture (M) and in the mixed culture of maize (M-MCS) and bean (B-MCS) in 2020 and 2021

Table 3. Yields of fresh matter (FM) in the maize monoculture (M) and in the mixed culture of maize (M-MCS) and bean (B-MCS) in 2020 and 2021 (t/ha), standard deviation (±SD), Tukey's HSD test.

Variant	Year	Maize			Bean		M, MCS (Maize+Bean) In Total		
		mean	±SD	HSD	mean	±SD	mean	±SD	HSD
M	2020	84.99	15.59	A	-	-	84.99	15.59	A
MCS	2020	79.96	15.05	A	16.23	5.31	96.19	16.22	A
M	2021	60.36	7.72	B	-	-	60.36	7.72	B
MCS	2021	48.53	12.92	A	34.55	6.41	83.08	11.14	A

Note: Data represent the mean and ±SD over 10 replications. Different letters show significant differences at P<0.05 among the crop variants M and MCS – at all times within a year.

Table 4. Yields of dry matter (DM) in the maize monoculture (M) and in the mixed culture of maize (M-MCS) and bean (B-MCS) in 2020 and 2021 (t/ha), standard deviation (±SD), Tukey's HSD test.

Variant	Year	Maize			Bean		M, MCS (Maize+Bean) In Total		
		mean	±SD	HSD	mean	±SD	mean	±SD	HSD
M	2020	31.86	4.88	A	-	-	31.86	4.88	A
MCS	2020	30.76	5.16	A	4.42	1.04	35.18	5.43	A
M	2021	26.54	3.10	B	-	-	26.54	3.10	A
MCS	2021	23.11	3.97	A	5.89	2.21	29.00	3.18	A

Note: Data represent the mean and ±SD over 10 replications. Different letters show significant differences at P<0.05 among the crop variants M and MCS – at all times within a year.

humid weather in 2021 as compared with 2020 (Table 1, Table 2). Lower temperatures might have a particularly negative influence on the biomass production. The weather conditions in 2020 were more favourable for M than for B, while the situation was reversed in 2021.

In their experiments with row spacing, Iptas and Acar [48] achieved DM yields in maize monocultures (M) ranging from 15.4 to 30.8 t/ha. Thus, maize yield recorded in the two experimental variants and years did not exhibit extremely high or low values. In contrast, Soe Htet et al. [46] recorded significantly lower values of biomass yield (DM) compared to our experiment. Soe Htet et al. [46] claim that the highest DM yield (14.56 t/ha) was recorded in the maize monoculture as compared

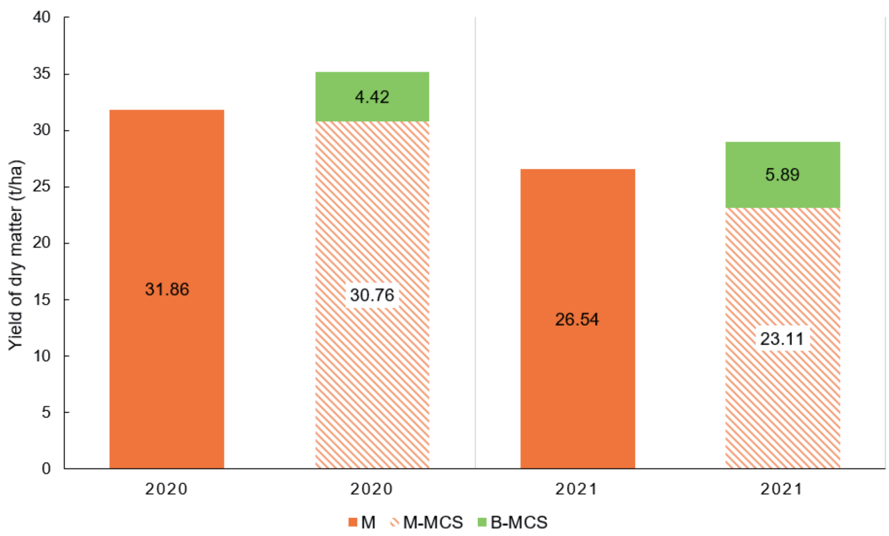

Fig. 5. Yields of dry matter (DM) in the maize monoculture (M) and in the mixed culture (MCS) of maize (M-MCS) and bean (B-MCS) in 2020 and 2021.

with MCS of maize with bean at various ratios. This difference in DM yields can be due to the harvest maturity stage. Li et al. [45] report that DM yields achieved in maize intercropped with bean ranged from 10.71 to 15.36 t/ha. In their experiments, DM yields of maize-soybean intercropping ranged from 11.39 to 18.03 t/ha. Based on 2-year experiments, Javanmard et al. [49] claim the DM yield in an MCS of maize and bean to be markedly higher (on average 13.2 t/ha) than in maize monocultures (on average 10.4 t/ha). It follows from the above-mentioned studies that the values recorded in this presented study do not show a more significant loss of DM yield in maize grown in the MCS, from which an assumption can be deduced that the chosen sowing rate of maize and bean in MCS was optimal as to its influence on biomass production.

Concentration of Heavy Metals in the Soil and Plant Biomass

The presence of HM in the shoot biomass of bean (B-MCS) and maize grown alone (M) and together with bean (M-MCS) was analysed. It was expected that in the case of HM, the ability of plants to accumulate them would be higher in legumes than in maize. This assumption was confirmed by Nielfa et al. [50] in their study. The assumption was confirmed by the results of this research as well (Table 5). The concentration of HM in bean (B) was significantly higher in the case of Cu, Hg, Zn and Fe than in maize grown as monoculture (M) or M-MCS in MCS during both years. In the case of Cr, the concentration in B was significantly higher only in 2020. Similarly, the content of Ni in B was significantly higher in MCS when compared to M only in 2021. The Cd concentration in the maize biomass did not significantly differ between B-MCS and maize variants (M-MCS and M) in the two years (Table 5).

Differences in the accumulation of HM in the above-ground biomass of maize were also assessed between the two maize variants (M-MCS and M). Only Fe was significantly higher ($P<0.05$) in Variant M when compared to M-MCS in 2020 (by 22.78%). However, the significance of this result was not confirmed in the following year 2021 (Table 5).

These results are not in agreement, for example, with Liu et al. [26], where the levels of Cd in the plant biomass were significantly higher when the crop plants were combined with legumes (Japanese clover) than when grown in the monoculture. Similarly, results published by Zhu et al. [54] showed that maize intercropped with soybean exhibited better extraction capacity of HM than monocropped maize. In the case of tomato plants, Cd level in fruits was below the maximum permissible concentration standard of the National Standard Agency in China when grown in monoculture, but above the level when co-planted with legumes [26]. Similarly, white lupin significantly increased the Fe concentration in oat tillers [55]. In another study, maize was grown alone and in the MCS with alfalfa *(Medicago sativa)*. Maize grown in MCS contained increased Fe levels

Table 5. Concentrations of heavy metals (mg/kg) in the above-ground biomass of bean (B-MCS), maize grown as monoculture (M) and in maize grown in the mixed cropping system (M-MCS).

Year	Heavy Metal	B-MCS	M-MCS	M	Reference values according to Oladejo et al. [51], Liu et al. [52] and Zhu et al. [53] for maize monoculture
2020	Cr	0.79±0.204^{b}	0.54±0.20^{ab}	0.37±0.05^{a}	3.22
	Cd	0.042±0.008^{a}	0.040±0.007^{a}	0.044±0.011^{a}	0.47
	Cu	5.85±2.31^{a}	3.204±0.75^{b}	2.69±0.40^{b}	5.19
	Ni	2.442±1.29^{a}	1.92±1.29^{a}	1.117±0.32^{a}	-
	Hg	0.0032±0.0004^{a}	0.002±0.0005^{b}	0.002±0.0002^{b}	0.010
	Zn	20±8.36^{a}	14.3±2.79^{ab}	12.6±2.23^{b}	32.51
	Fe	67.23±5.84^{a}	39.63±4.66^{c}	51.32±3.60^{b}	-
2021	Cr	0.58±0.19^{A}	0.43±0.10^{A}	0.69±0.25^{A}	3.22
	Cd	0.059±0.020^{A}	0.047±0.012^{A}	0.048±0.011^{A}	0.47
	Cu	5.051±0.627^{A}	2.994±0.43^{B}	2.756±0.52^{B}	5.19
	Ni	1.274±0.34^{A}	0.323±0.161^{B}	0.448±0.316^{B}	-
	Hg	0.0056±0.0017^{A}	0.0021±0.0007^{B}	0.0021±0.0007^{B}	0.010
	Zn	18.4±3.58^{A}	13.1±1.18^{B}	11.75±1.35^{B}	32.51
	Fe	123.43±44.58^{A}	55.59±5.98^{B}	63.11±19.28^{B}	-

Note: Data represent the mean±standard deviation over 10 replications. Different letters (lower case for 2020 and capitals for 2021) show significant differences at $P<0.05$ (ANOVA, Tukey's test) among the crop variants and within individual HM.

when compared to crops grown alone (from 171.9-175.0 to 180.6-184.0 μg/g) [56]. Similarly, the Fe concentration was significantly higher in tillers of oat intercropped with white lupin [55]. The acquisition of Fe is affected by multiple factors, such as soil microbiome, especially siderophore-producing plant growth-promoting bacteria which enhance plant iron nutrition [57] or the presence of crops producing a high amount of coumarin. Coumarin seems to play an important role in the acquisition of Fe by plants. In particular, coumarin biosynthesis pathways are highly responsive to Fe deficiency and have also been described to be regulated by FIT (Fer-like iron deficiency-induced transcription factor) [58, 59]. In the case of maize intercropped with Japanese clover herb, the concentration of Cd, Cr and Cu in the above-ground biomass of maize was reduced by 24.69, 54.5, and 20.76 mg/kg DM, respectively, when compared to maize grown alone [60]. The experiment took place in a field that had not been previously contaminated with HM. Therefore, in contaminated soils, the concentration of HM in shoot biomass of crops would probably be higher. According to Zhang et al. [27], the presence of Cd in plant shoots was linearly dependent on its content in the soil.

Apart from the above-mentioned comparison, Table 5 provides reference values of HM contents from some scientific studies. The studies dealt with analysing the capacity of maize to bind HM when it is grown in an environment contaminated by HM. In all cases, values measured in this experiment do not suggest that the field experiment was conducted on contaminated soil. In any case, they point to the fact that maize in MCS exhibited higher accumulation of HM (except for Fe) as compared with maize in monoculture, and the concentration of HM approached reference values chosen in the presented research study.

The initial content of HM in the soil on the experimental site before the establishment of the field experiment is presented in Table 6. A comparison with internationally comparable geochemical background values [61] shows that no heavy metal occurred on the experimental site at an elevated concentration. Only in the case of Hg, a difference of 0.039 mg/kg was recorded between the reference value and the soil content. This was also confirmed by the measured values of HM concentrations presented in Table 5, which did not exceed reference values of HM content in the biomass of maize grown on contaminated plots but were instead at least 40% lower in all cases.

BAC was calculated to assess the phytoremediation potential of the MCS combining bean and maize (Tables 7 and 8). BAC is often used to evaluate the capability for HM uptake by plants and the metal transport to the above-ground plant biomass [62]. If the HM BAC is higher than 1 (Table 8), the plant readily transports it from the soil into the above-ground parts of the plant body [63]. The mobility of HM in the soil depends on various factors, such as precipitation, soil pH or organic matter in the soil [64, 65]. Similarly, the application of biochar significantly affected the effectiveness of Cu, Cd, Pb and Zn immobilization in the soil [66]. In order to have a more detailed comparison, a reference limit was chosen again, namely the BAC value from Liu et al. [52] where the authors dealt with the issue of maize contamination by HM during irrigation with wastewater. Reference BAC values from the environment contaminated with heavy metals were always higher than BAC values recorded in the presented experiment. BAC values calculated in the presented experiment indicate that both crops fall into the category of low accumulator plants for most HM (Cr, Ni, Hg and Fe) and moderate accumulator plants for Cd, Cu and Zn. The finding also corresponds to reference values indicating that maize fell into the category of moderate accumulator plants for the majority of HM with the exception of Cr.

It seems that the green parts of maize accumulate HM more than the other ones (grains) [53]. In the presented study, BAC was higher than 1 only in the case of Cd accumulated by maize grown as monoculture in 2021 (Table 7). However, this trend was not observed in 2020, and BAC of Cd for M was 0.38. The difference could have been caused by different meteorological conditions in 2020 and 2021. In the case of maize grown alone in the presence of elevated Cd (5 mg/kg and 10 mg/kg), the BAC factor for maize leaves was 1.582 and 1.217 for 5 and 10 mg/kg of Cd, respectively [62].

The HM least transported by plants from the soil to the above-ground biomass of maize was Fe. BAC for Fe reached a maximum for M in 2021 (0.0019) and

Table 6. Presence of heavy metals in the soil at the experimental site.

HM	mg/kg							
	Cr	Cd	Cu	Ni	Pb	Hg	Zn	Fe
Mean±SD	39.3±0.1	0.117±0.009	21.33±0.4	27.23±0.3	11.7±0.3	0.059±0.001	61.1±0.2	32 800±2523.9
Geochemical background value (Guan et al. 2014)	67.30	0.10	22.50	31.00	21.00	0.02	65.40	-

Note: Data represent the mean±standard deviation over 6 replications.

Table 7. Bioaccumulation factor of Phaseolus vulgaris (B), Zea mays grown in monoculture (M) and in a mixed cropping system with bean (M-MCS); values are presented as means.

Year	BAC	Cr	Cd	Cu	Ni	Pb	Hg	Zn	Fe
2020	B-MCS	0.020	0.35	0.274	0.089	0.025	0.054	0.33	0.0025
	M	0.009	0.38	0.126	0.041	0.015	0.035	0.21	0.0016
	M-MCS	0.014	0.34	0.150	0.070	0.015	0.034	0.23	0.0012
2021	B-MCS	0.017	0.50	0.237	0.047	0.026	0.094	0.30	0.0038
	M	0.017	1.06	0.129	0.016	0.002	0.035	0.19	0.0019
	M-MCS	0.003	0.10	0.020	0.006	0.014	0.012	0.02	0.0002
Liu et al. [52]	Maize monoculture	0.080	0.71	0.860	-	0.190	-	0.88	-

Table 8. Evaluation of BAC values according to Malayeri et al. [67].

BAC	Category
<0.01	Non-accumulator plants
0.01-0.1	Low accumulator plants
0.1-1.0	Moderate accumulator plants
1.0-10.0	High accumulator plants

for M-MCS in 2020 (0.0012). These results are similar to those reported by An et al. [60] where maize was intercropped with Japanese clover. The lowest BAC was recorded for Fe in maize grown in monoculture (M) and in M-MCS. According to the results of the presented study, it was not obvious whether M accumulates HM in its above-ground biomass. BAC was higher for maize grown alone during both years in the case of Cd, Hg and Fe, and for Cu it was lower in 2020 (Table 7). In An et al. (2011), BAC of all observed HM (Cd, Pb, Cr, Cu and Fe) was higher in the case of maize grown alone as compared with the intercropped maize and bean.

In the case of Cu, Ni, Pb, Hg, Zn and Fe, bean accumulated HM more readily than maize grown alone or in MCS. However, similarly to the case of maize, BAC of none of the HM accumulated by bean exceeded the value of 1 (Table 7). Thus, for most HM, bean and maize were either low or moderate accumulator plants. The cumulative presence of HM on fields with the maize monoculture and MCS composed of maize and bean was calculated from the yield and HM concentrations in plant biomass (Annex, Fig. A3). The overall HM levels in crops harvested from one hectare are strictly linked to the amount of metals in the soil. The concentration of HM in the harvested crops was higher in the biomass from MCS (M-MCS+B-MCS). The overall quantity of HM in the harvested biomass was Fe>Zn>Cu>Ni>Cr>Cd>Hg (Fig. A3). The amount of HM in the system should be considered if the biomass is to be used in methane production, the reason being possible toxicity of HM to microbes participating in the processes of anaerobic digestion [25]. It is also necessary to bear in mind that the severity of metal inhibition depends on various factors such as the concentration of HM in a soluble, ionic form in the substrate solution, type of metal species, bacterial genera and amount of biomass in the digester [24]. According to Alrawashdeh et al. [68], the toxicity of HM can be arranged in increasing order: Cu>Ni>Pb>Cr>Zn>Fe, which leads to the rapid poisoning of active microorganisms.

Monitoring the content of HM in the biomass of crops grown as feedstock for biogas plants is important due to the toxicity of some HM in the process of anaerobic digestion. According to Golub et al. [25], the toxicity of HM for anaerobic digestion can be classified in the following decreasing order: Cu>Ni>Pb>Cr>Zn>Fe. However, a theoretical potentially beneficial effect of some HM on the growth of crops should be considered, too. Some HM (Cu, Ni, Zn, etc.) are crucial for the proper functioning of many enzymes important in the processes of anaerobic digestion [24, 25]. Wang et al. [24] found that the biogas yield of maize grown in the soil slightly polluted with Cd was higher than in the soil without Cd contamination. Changes in the acidogenesis stage led to problems in methanogenesis due to the limited presence of methane-producing organisms. Zhang et al. [28] claim that the severity of the effect on anaerobic digestion depends on the type of HM. At Cr dosages higher than 5 mg/L, Cr^{III} caused a more severe inhibition of the acidogenesis stage than Cd did. Similarly, in Golub et al. [25], a dosage of Cr (0.519 mg/L) reduced the daily production of methane from the olive mill waste by 56%. In the same study, the effect of Zn on the anaerobic digestion of olive mill waste was assessed. Addition of Zn in a concentration of 0.072-0.29 mg/L reduced biogas production and doses higher than 0.29 mg/L stopped it. It is therefore possible to state that the issue of HM concentration in the plant biomass fed to a biogas-generating fermenter has not been described in all possible aspects yet. The potential influence of HM can be both negative [69] and positive [24], which shows the importance of monitoring their concentration in the biomass of crops used for energy purposes.

Theoretical Methane Yield

TMY was calculated from values listed in Annex Table A1 to assess differences in methane yields obtained from the maize monoculture (M) and from the MCS composed of maize (M-MCS) and bean (B-MCS) (Fig. 6). In the two years of this experiment, no significant difference was recorded between Variant M (0.386 and 0.375 m^3/kg_{VS} in 2020 and 2021, respectively) and Variant M-MCS (0.397 and 0.399 m^3/kg_{VS} in 2020 and 2021, respectively). However, when compared with bean, TMY was significantly higher for both maize variants only in 2020. Compared with bean, TMY was higher only for M-MCS in 2021. The results of the research showed that biomass from M-MCS exhibited a higher TMY than biomass obtained from M in both years 2020 and 2021. In 2020, TMY of maize grown in monoculture was 2.86% lower than in MCS while in

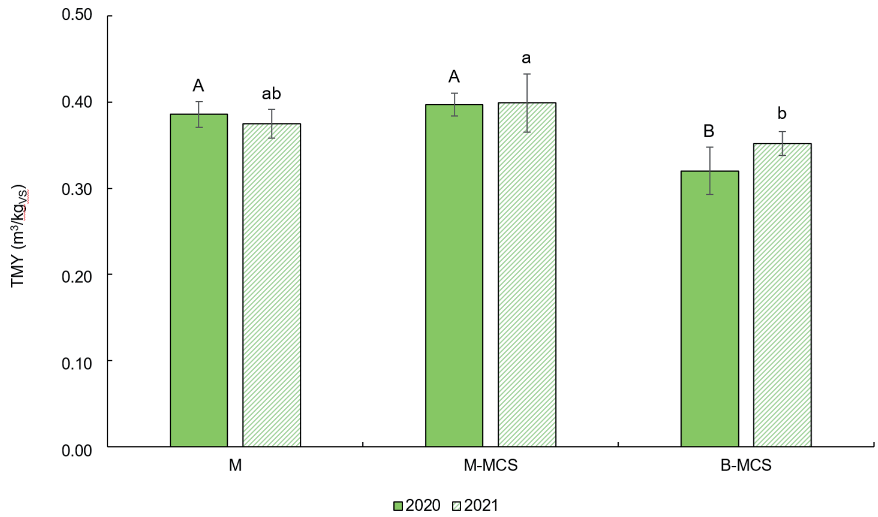

Fig. 6. Theoretical methane yield (TMY) of biomass obtained from maize monoculture (M) and mixed cropping system (MCS) composed of maize (M-MCS) and bean (B-MCS). Data in columns represent the mean±standard deviation over 10 replications. Different letters (uppercase for 2020 and lowercase for 2021) show significant differences at $P<0.05$ (ANOVA, Tukey's test) between the crop variants.

Table 9. Examples of methane yield from energy plants and crop residues presented in the literature. Green colour is used for the values of relative methane yields higher than the reference methane yield in maize (351.0 NL/kgVS).

Crop	Methane yield (NL/kg)	Relative methane yield (maize 351 NL/kgVS) = 100%	Reference
Maize – reference methane yield	351*	100.0	[74, 75]
Maize in monoculture	380**	108.4	TMY
Maize in MCS with bean	398**	113.4	TMY
Bean in MCS with maize	336**	95.7	TMY
Pea	390	111.1	[76]
Clover grass	290-390 (340)***	96.9	[77]
Meadow clover	300	85.5	[78]
Hemp	355-377 (366)***	104.3	[79]
Sunflower	400	114.0	[80]
Jerusalem artichoke	370	105.4	[81]
Lucerne	340-350 (345)***	98.3	[82]

Note: *Methane yield values were calculated as a mean from data presented in both publications. **Methane yield values were calculated as a mean from data of a 2-year experiment (2020 and 2021). ***Methane yield values were calculated as a mean (in parentheses) from minimum and maximum values presented in the publication. The reference methane yield of 351 NL/kgVS is expressed as normal liters (NL) per kilogram of volatile solids (VS), where NL corresponds to gas volume at standard conditions (0°C and 101.325 kPa) and VS represents the biodegradable organic fraction of biomass.

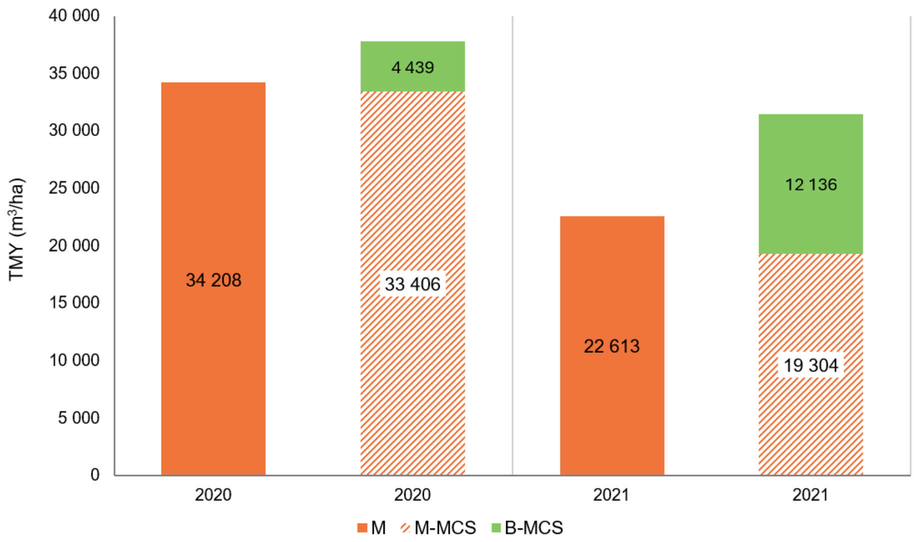

Fig. 7. Theoretical methane yield (TMY) of dry biomass obtained from maize monoculture (M) and mixed cropping system (MCS) composed of maize (M-MCS) and bean (B-MCS) converted to m^3/ha.

Table 10. Theoretical methane yield (TMY) of fresh biomass (FM) obtained from maize monoculture (M) and mixed cropping system (MCS) composed of maize (M-MCS) and bean (B-MCS) converted to m^3/ha; standard deviation (±SD), Tukey's HSD test.

Variant	Year	Maize (m^3/ha)			Bean (m^3/ha)		Maize+Bean (m^3/ha)		
		mean	±SD	HSD	mean	±SD	mean	±SD	HSD
M	2020	34 207.99	3 027.50	A	-	-	34 207.99	3 027.50	A
MCS		33 406.11	3 688.91	A	4 439.48	958.676	37 845.59	3 315.59	A
M	2021	22 612.52	3 034.21	A	-	-	22 612.52	3 034.21	B
MCS		19 303.91	5 336.56	A	12 136.10	2 222.87	31 440.02	4 513.39	A

Note: Data represent the mean and ±SD over 10 replications. Different letters show significant differences at $P<0.05$ between the crop variants M and MCS at all times within a year.

2021 it was higher by 6.06% (Fig. 6, Table A1). However, these differences were not significant.

Compared with values reported in other studies, the TMY values calculated in this study for both M and M-MCS were lower. Triolo et al. [40] reported TMY values of 0.443 and 0.445 m^3/kg_{VS} for maize monoculture, while Kintl et al. [70] reported a value of 0.427 m^3/kg_{VS}. Intriguingly, Li et al. [71] calculated TMY for corn stover biomass (0.455 m^3/kg_{VS}), which was also slightly higher when compared to the results of the present study. The differences might have been caused by different conditions in which the crops were grown such as the share of organic matter in the soil, availability of mineral nutrients or water. Kintl et al. [16] claim, for example, that TMY of maize grown in the MCS with white lupin (*Lupinus albus*) was lower than in lupin grown as monoculture.

The measured values indicate that MCS can affect and in the case of some parameters even enhance the quality of biomass intended for biomethane production. It, however, also depends on the intercrop used in the MCS. Kintl et al. [16, 72] found, for example, that an MCS of maize with Jerusalem artichoke (*Helianthus tuberosus*) and lupin (*Lupinus albus*) did not exhibit TMY different from that of maize monoculture. At the same time, the actual yield of biomethane was significantly decreased in the MCS. Also, as mentioned by Rengganman et al. [73], it should be considered that TMY is calculated based on the composition of organic substances and assumes 100% decomposition of matter, including difficult-to-decompose materials. Therefore, TMY may differ from the actual production of biogas (methane). A comparison of calculated TMY values with values published by other authors is presented in Table 9.

In 2020, TMY converted to unit area (m^3/ha) was 9.61% higher in MCS than in the maize monoculture (M). The difference was, however, not significant (Table 9). The share of bean in total TMY/m^3/ha of the MCS was only 11.73%. In 2021, MCS exhibited TMY/m^3/ha 28.07% higher than the maize monoculture (M) (Fig. 7) with the difference being significant (Table 9).

The proportion of bean in total TMY/m^3/ha of MCS was 38.06%. TMY/m^3/ha of MCS in 2021 was 16.92% lower than in 2020. TMY/m^3/ha of the maize monoculture (M) in 2021 was 33.9% lower than in 2020. A significant difference was recorded between TMY/m^3/ha of the maize monoculture (M) and TMY/m^3/ha of maize grown in the M-MCS in 2021 (Table 10). Mixed cropping systems containing legumes are particularly interesting due to the higher amount of nitrogen compounds in total biomass. Nitrogen is an important component of biomass used for methane production, and it is usually scanty in biomass obtained from the maize monoculture, which can lead to unstable anaerobic digestion [83].

The results indicate that weather conditions in 2021 were more suitable for beans than they were for maize (Figs. 4 and 7, Tables 1 and 2), which was reflected in both the yields and the values of TMY of TMY/m^3/ha.

Conclusions

The capacity to accumulate HM was tested in the mixed cropping system composed of common bean and maize. The results show that the presence of the legume did not significantly affect the efficiency of HM accumulation in the above-ground parts of maize. None of the variants showed a rate of HM accumulation in its shoots that would have indicated its suitability for phytoremediation processes. As for biomass production, biomass from the mixed cropping system exhibited higher yields than biomass from the maize monoculture. The hypothesis concerning HM accumulation was confirmed only partially. Bean grown in the mixed cropping system accumulated higher concentrations of several HM than maize, but intercropping had only a limited effect on HM concentrations in the maize biomass when compared with maize monoculture. Significant differences between maize grown in the monoculture and maize grown in the mixed cropping system were found only for Fe in 2020. Therefore, under the given soil conditions, the mixed cropping system did not markedly enhance the HM accumulation in maize biomass and did not indicate a clear phytoremediation effect.

However, TMY can be lower in the MCS than in the maize monoculture. TMY obtained from the fresh matter and converted to a unit area was in both years higher in the MCS than TMY in the maize monoculture, with the difference being significant only in the second year of the experiment. The ratio of crops in the biomass of MCS changed depending on meteorological conditions.

The proposed hypotheses were supported only partially. The mixed cropping system positively affected biomass production, but not consistently in all years and biomass parameters. The hypothesis related to methane production was supported only when TMY was evaluated on an area basis, while the hypothesis related to HM accumulation was supported to a limited extent only. These findings suggest that under common agricultural conditions with low background metal concentrations, maize–bean intercropping can be considered a promising strategy for increasing biomass production and methane yield per hectare, whereas its effect on HM accumulation remains limited.

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Conflict of Interest

The authors declare no conflict of interest.

Credit Authorship Contribution Statement

Antonín Kintl: conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing – original draft, supervision, project administration, funding acquisition.

Ján Smeringai: conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing – original draft.

Martin Brtnický: validation, formal analysis.

Julie Sobotková: conceptualization, methodology, validation, formal analysis.

Igor Huňady: methodology, validation, formal analysis, investigation, data curation, visualization.

Jiří Holátko: validation.

Tomáš Vítěz: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, writing – review and editing, supervision.

Jakub Elbl: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, writing – review and editing, visualization.

References

1. MAKOVÁ J., JAVOREKOVÁ S., ELBL J., MEDO J., HRICÁKOVÁ N., KOVÁČIK P. Impact of vermicompost on biological indicators of the quality of soil under maize in a greenhouse experiment. Journal of Elementology, **24** (1), 319, **2019**.
2. BOARDMAN J. How much is soil erosion costing us? Geography **106** (1), 32, **2021**.
3. ORTÍZ SANDOVAL P., AGUILERA-GÓMEZ M., KOSTKA A., GRUSZECKA-KOSOWSKA A. Cumulative exposure of xenobiotics of emerging concern from agrifood under the One Health approach (XENOBAC4OH). EFSA Journal, **23** (S1), e231104, **2025**.
4. ELBL J., ZÁHORA J. The comparison of microbial activity in rhizosphere and non-rhizosphere soil stressed by drought. In Polák O., Cerkal R., Škarpa P. (Eds.)

MendelNet 2014: Proceedings of International Ph.D. Students Conference. Brno, Czech Republic: Mendel University in Brno, pp. 234, **2015**.
5. HE Z., SHENTU J., YANG X., BALIGAR V.C., ZHANG. T., STOFFELLA P.J. Heavy Metal Contamination of Soils: Sources, Indicators and Assessment. Journal of Environmental Indicators, **9**, 17, **2015**.
6. SHAH A., TYAGI S., SINGH R.K., TIWARI K.K., PRASAD G.G., KALIA S., KUMAR R., RAI V. Biotic and abiotic stress management in agriculture: The transformative role of nanotechnology. Plant Nano Biology, **14**, 100202, **2025**.
7. BOSTOCK R.M., PYE M.F., ROUBTSOVA T.V. Predisposition in Plant Disease: Exploiting the Nexus in Abiotic and Biotic Stress Perception and Response. Annual Reviews Phytopathology, **52** (1), 517, **2014**.
8. MULLER A. Sustainable agriculture and the production of biomass for energy use. Climate Change, **94** (3-4), 319, **2009**.
9. LI C., HOFFLAND E., KUYPER T.W., YU Y., ZHANG C., LI H., ZHANG F., van der WERF, W. Syndromes of production in intercropping impact yield gains. Nature Plants, **6** (6), 653, **2020**.
10. SHAHZAD K., MAIER S., NARODOSLAWSKY M. Biogas Production from Intercropping (Syn-Energy). Chemical Engineering Transactions, **39**, 1753, **2014**.
11. TAKASU A.T., ARF O., MEIRELLES F.C., DE LIMA MILHOSSI E.N., PORTUGAL J.R., DE SOUZA BUZO F., KANEKO F.H., FERREIRA RODRIGUES R.A. Intercropping of grasses or legume species in maize crop in the cerrado. Revista Brasileira de Milho e Sorgo, **19**,18, **2020**.
12. BROOKER R.W., BENNETT A.E., CONG W.F., DANIELL T.J., GEORGE T.S., HALLETT P.D., HAWES C., IANNETTA P.P.M., JONES H.G., KARLEY A.J., LI L., MCKANZIE B.M., PAKEMAN R.J., PATERSON E., SCHOB C., SHEN J., SQUIRE G., WATSON C.A., THANG C., ZHANG F., ZHANG J., WHITE P.J. Improving intercropping: a synthesis of research in agronomy, plant physiology and ecology. New Phytologist, **206** (1), 107, **2014**.
13. RECKLING M., BERGKVIST G., WATSON C.A., STODDARD F.L., ZANDER P.M., WALKER R.L., PRISTERI A., TONCEA I., BACHINGER J. Trade-Offs between Economic and Environmental Impacts of Introducing Legumes into Cropping Systems. Frontiers in Plant Sciences, **7** (669), 1, **2016**.
14. DUCHENE O., VIAN J.-F., CELETTE F. Intercropping with legume for agroecological cropping systems: Complementarity and facilitation processes and the importance of soil microorganisms. A review. Agriculture, Ecosystems & Environment, **240**, 148, **2017**.
15. KINTL A., ELBL J., LOŠÁK T., VAVERKOVÁ M.D., NEDĚLNÍK J. Mixed Intercropping of Wheat and White Clover to Enhance the Sustainability of the Conventional Cropping System: Effects on Biomass Production and Leaching of Mineral Nitrogen. Sustainability, **10** (10), 3367, **2018**.
16. KINTL A., VÍTĚZ T., ELBL J., VÍTĚZOVÁ M., DOKULILOVÁ T., NEDĚLNÍK J., SKLÁDANKA J., BRTNICKÝ M. Mixed Culture of Corn and White Lupine as an Alternative to Silage Made from Corn Monoculture Intended for Biogas Production. BioEnergy Research, **12** (3), 694, **2019**.
17. ATIENZA S.G., RUBIALES D. Legumes in sustainable agriculture. Crop & Pasture Science, **68** (11), i, **2017**.
18. THILAKARATHNA M.S., PAPADOPOULOS Y.A., RODD A.V., GRIMMETT M., FILLMORE S.A.E., CROUSE M., PRITHIVIRAJ B. Nitrogen fixation and transfer of red clover genotypes under legume–grass forage based production systems. Nutrient Cycling in Agroecosystems, **106** (2), 233, **2016**.
19. SUÁREZ J.C., ANZOLA J.A., CONTRERAS A.T., SALAS D.L., VANEGAS J.I., URBAN M.O., BEEBE S.E., RAO I.M. Agronomic performance evaluation of intercropping two common bean breeding lines with a maize variety under two types of fertilizer applications in the Colombian Amazon region. Agronomy, **12** (2), 307, **2022**.
20. VITEZ T., DOKULILOVA T., VITEZOVA M., ELBL J., KINTL A., KYNICKY J., HLADKY J., BRTNICKY M. The Digestion of Waste from Vegetables and Maize Processing. Waste and Biomass Valorization, **11** (6), 2467, **2020**.
21. SOHA T., PAPP L., CSONTOS C., MUNKÁCSY B. The importance of high crop residue demand on biogas plant site selection, scaling and feedstock allocation – A regional scale concept in a Hungarian study area. Renewable and Sustainable Energy Reviews, **141**, 110822, **2021**.
22. WANG J., CAI Y., YANG J., ZHAO X. Research trends and frontiers on source appointment of soil heavy metal: a scientometric review (2000-2020). Environmental Science and Pollution Research, **28** (38), 52764, **2021**.
23. VAVERKOVÁ M.D., ZLOCH J., ADAMCOVÁ D., RADZIEMSKA M., VYHNÁNEK T., TROJAN V., WINKLER J., ĐORĐEVIĆ B., ELBL J., BRTNICKÝ M. Landfill Leachate Effects on Germination and Seedling Growth of Hemp Cultivars (*Cannabis Sativa* L.). Waste Biomass Valorization, **10** (2), 369, **2019**.
24. WANG S., WANG J., LI J., HOU Y., SHI L., LIAN C., SHEN Z., CHEN Y. Evaluation of biogas production potential of trace element-contaminated plants via anaerobic digestion. Ecotoxicology and Environmental Safety, **208**, 111598, **2021**.
25. GOLUB N., SHYNKARCHUK A., KOZLOVETS O., XINHUA S. Influence of heavy metals on the process of anaerobic fermentation of biomass by the consortia of anaerobic microorganisms. Innovative Biosystems and Bioengineering, **4** (4), 211, **2020**.
26. LIU L., ZHANG Q., HU L., TANG J., XU L., YANG X., YONG J.W.H., CHEN X. Legumes Can Increase Cadmium Contamination in Neighboring Crops. PLoS ONE, **7** (8), e42944, **2012**.
27. ZHANG H., TIAN Y., WANG L., ZHANG L., DAI L. Ecophysiological characteristics and biogas production of cadmium-contaminated crops. Bioresource Technology, **146**, 628, **2013**.
28. ZHANG L., YANG H., WEI D., CHEN Z., WANG Q., SONG Y., MA Y., ZHANG H. Tolerance of anaerobic digestion sludge to heavy metals: COD removal, biogas production, and microbial variation. Journal of Water Process Engineering, **55**, 104157, **2023**.
29. HUNCE S.Y., CLEMENTE R., BERNAL M.P. Energy production potential of phytoremediation plant biomass: Helianthus annuus and Silybum marianum. Industrial Crops and Products, **135**, 206, **2019**.
30. BERNAL M.P., GRIPPI D., CLEMENTE R. Potential of the Biomass of Plants Grown in Trace Element-Contaminated Soils under Mediterranean Climatic Conditions for Bioenergy Production. Agronomy, **11** (9), 1750, **2021**.
31. TOKER P., CANCI H., TURHAN I., İSÇİ A., SCHERZINGER M., KORDROSTAMI M., YOL E. The

advantages of intercropping to improve productivity in food and forage production – a review. Plant Production Science, **27** (3), 155, **2024**.
32. CHEN C., WESTCOTT M., NEILL K., WICHMAN D., KNOX M. Row Configuration and Nitrogen Application for Barley–Pea Intercropping in Montana. Agronomy Journal, **96** (6), 1730, **2004**.
33. WORKAYEHU T., WORTMANN C.S. Maize–Bean Intercrop Weed Suppression and Profitability in Southern Ethiopia. Agronomy Journal, **103** (4), 1058, **2011**.
34. PRETTY J., BHARUCHA Z.P. Sustainable intensification in agricultural systems. Annals of Botany, **114** (8), 1571, **2014**.
35. KUBÍKOVÁ Z., HUTYROVÁ H., SMEJKALOVÁ H., KINTL A., ELBL J. Application of extended BBCH scale for studying the development of *Phacelia tanacetifolia* Benth. Annals of Applied Biology, **181** (3), 332, **2022**.
36. KINTL A., SMERINGAI J., SOBOTKOVÁ J., HUŇADY I., BRTNICKÝ M., HAMMERSCHMIEDT T., ELBL J. Mixed cropping system of maize and bean as a local source of N-substances for the nutrition of farm animals. European Journal of Agronomy, **154**, 127059, **2024**.
37. LOUČKA R., LANG J., JAMBOR V., NEDĚLNÍK J., TŘINÁCTÝ J., TYROLOVÁ Y., KUČERA J. Verified methodical process of obtaining and processing the values in the national system of evaluation of silage corn hybrids, The certified methodology, 1st Ed.; Agricultural Research Institute Ltd.: Troubsko, Czech Republic, pp. 20, **2014** [In Czech].
38. CSN EN 11885, Water quality - Determination of selected elements by inductively coupled plasma optical emission spectrometry (ICP-OES) (ISO 11885:2007) 2009. Available online: https://standards.iteh.ai/catalog/standards/sist/92da1257-ba76-4915-8382-fe8f1b99f6e4/sist-en-iso-11885-2009 (accessed on 10 June 2022).
39. HELRICH K. Official methods of analysis of the Association of Official Analytical Chemists, 15th ed.;.AOAC: Arlington, Virginia, USA, **1990**.
40. LESTEUR M., BELLON-MAUREL V., GONZALEZ C., LATRILLE E., ROGER J.M., JUNQUA G., STEYER J.P. Alternative methods for determining anaerobic biodegradability: A review. Process Biochemistry, **45** (4), 431, **2010**.
41. TRIOLO J.M., SOMMER S.G., MØLLER H.B., WEISBJERG M.R., JIANG X.Y. A new algorithm to characterize biodegradability of biomass during anaerobic digestion: influence of lignin concentration on methane production potential. Bioresource Technology, **102** (20), 9395, **2011**.
42. PHAM C.H., TRIOLO J.M., CU T.T.T., PEDERSEN L., SOMMER S.G. Validation and Recommendation of Methods to Measure Biogas Production Potential of Animal Manure. Asian-Australas Journal of Animal Science, **26** (6), 864, **2013**.
43. AMIN H., ARAIN B.A., JAHANGIR T.M., ABBASI M.S., AMIN F. Accumulation and distribution of lead (Pb) in plant tissues of guar (*Cyamopsis tetragonoloba* L.) and sesame (*Sesamum indicum* L.): profitable phytoremediation with biofuel crops. Geology Ecology and Landscape, **2** (1), 51, **2018**.
44. KARASU A., OZ M., BAYRAM G., TURGUT IL. The effect of nitrogen levels on forage yield and some attributes in some hybrid corn (*Zea mays indentata* Sturt.) cultivars sown as second crop for silage corn. African Journal of Agricultural Research, **4** (3), 166, **2009**.
45. LI J., WEN, X., YANG J., YANG W., XIN Y., ZHANG L., LIU H., HE Y., YAN Y. Effects of Maize Varieties on Biomass Yield and Silage Quality of Maize–Soybean Intercropping in the Qinghai–Tibet Plateau. Fermentation, **8** (10), 542, **2022**.
46. SOE HTET M.N., WANG H., YADAV V., SOMPOUVISETH T., FENG B. Legume Integration Augments the Forage Productivity and Quality in Maize-Based System in the Loess Plateau Regional. Sustainability, **14** (10), 6022, **2022**.
47. KINTL A., HUŇADY I., VÍTĚZ T., BRTNICKÝ M., SOBOTKOVÁ J., HAMMERSCHMIEDT T., VÍTĚZOVÁ M., HOLÁTKO J., SMUTNÝ V., ELBL J. Effect of Legumes Intercropped with Maize on Biomass Yield and Subsequent Biogas Production. Agronomy, **13** (11), 2775, **2023**.
48. IPTAS S., ACAR A.A. Effects of hybrid and row spacing on maize forage yield and quality. Plant, Soil and Environment, **52** (11), 515, **2006**.
49. JAVANMARD A., NASAB A., JAVANSHIR A., MOGHADDAM M., JANMOHAMMADI H. Forage yield and quality in intercropping of maize with different legumes as double-cropped. International Journal of Food Agriculture and Environment, **7** (1), 163, **2009**.
50. NIELFA A., CANO R., FDZ-POLANCO M. Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. Biotechnology Reports, **5**, 14, **2014**.
51. OLADEJO N.A., ANEGBE B., ADENIYI O. Accumulation of Heavy Metals in Soil and Maize Plant (*Zea mays*) in the Vicinity of Two Government Approved Dumpsites in Benin City, Nigeria. Asian Journal of Chemical Sciences, **3** (3), 1, **2017**.
52. LIU W.-H., ZHAO J.-Z., OUYANG Z.-Y., SÖDERLUND L., LIU G.-H. Impacts of sewage irrigation on heavy metal distribution and contamination in Beijing, China. Environment International, **31** (6), 805, **2005**.
53. ZHU S.-Y., MA X., GUO R., AI S., LIU B., ZHANG W., ZHANG Y. A Field Study on Heavy Metals Phytoattenuation Potential of Monocropping and Intercropping of Maize and/or Legumes in Weak Alkaline Soils. International Journal of Phytoremediation, **18** (10), 1014, **2016**.
54. BOADI N., OKYERE H., BADU M., MENSAH J., OWUSU-APPIAH I. Effect of Mercury on the Proximate Composition of Maize (*Zea mays* L.). Journal of Agricultural Sciences and Technology, **3**, 487, **2013**.
55. WICHE O., SZÉKELY B., KUMMER N.-A., MOSCHNER C., HEILMEIER H. Effects of intercropping of oat (*Avena sativa* L.) with white lupin (*Lupinus albus* L.) on the mobility of target elements for phytoremediation and phytomining in soil solution. International Journal of Phytoremediation, **18** (9), 900, **2016**.
56. NASAR J., SHAO Z., GAO Q., ZHOU X., FAHAD S., LIU S., LI C., BANDA S.K.J., KGORUTLA L.E., DAWAR, K.M. Maize-alfalfa intercropping induced changes in plant and soil nutrient status under nitrogen application. Archives of Agronomy and Soil Sciences, **68** (2), 151, **2022**.
57. SAEED Q., XIUKANG W., HAIDER F.U., KUČERIK J., MUMTAZ M.Z., HOLATKO J., NASEEM M., KINTL A., EJAZ M., NAVEED M., BRTNICKY M., MUSTAFA A. Rhizosphere Bacteria in Plant Growth Promotion, Biocontrol, and Bioremediation of Contaminated Sites: A Comprehensive Review of Effects and Mechanisms.

International Journal of Molecular Sciences, **22** (17), 10529, **2021**.
58. CLEMENS S., WEBER M. The essential role of coumarin secretion for Fe acquisition from alkaline soil. Plant Signaling and Behaviour, **11** (2), **2016**.
59. STASSEN M.J.J., HSU S.-H., PIETERSE C.M.J., STRINGLIS I.A. Coumarin Communication Along the Microbiome–Root–Shoot Axis. Trends in Plant Science, **26** (2), 169, **2021**.
60. AN L., PAN Y., WANG Z., ZHU C. Heavy metal absorption status of five plant species in monoculture and intercropping. Plant Soil, **345** (1-2), 237, **2011**.
61. GUAN Y., SHAO C., JU M. Heavy Metal Contamination Assessment and Partition for Industrial and Mining Gathering Areas. International Journal of Environmental Research and Public Health, **11** (7), 7286, **2014**.
62. TIAN Y., ZHANG H., GUO W., CHEN Z.S., WEI X., ZHANG L., HAN L., DAI L. Assessment of the phytoremediation potential of bioenergy crop maize (*Zea mays*) in soil contaminated by cadmium: Morphology, photosynthesis and accumulation. Fresenius Environmental Bulletin, **21** (11), 3575, **2012**.
63. FITZ W.J., WENZEL W.W. Arsenic transformations in the soil–rhizosphere–plant system: fundamentals and potential application to phytoremediation. Journal of Biotechnology, **99** (3), 259, **2002**.
64. WIECZOREK J., BARAN A., BUBAK A. Mobility, bioaccumulation in plants, and risk assessment of metals in soils. Science of The Total Environment, **882**, 163574, **2023**.
65. ZENG F., ALI S., ZHANG H., OUYANG Y., QIU B., WU F., ZHANG G. The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. Environmental Pollution, **159** (1), 84, **2011**.
66. RADZIEMSKA M., GUSIATIN Z.M., MAZUR Z., HAMMERSCHMIEDT T., BĘŚ A., KINTL A., GALIOVA M.V., HOLATKO J., BLAZEJCZYK A., KUMAR V., BRTNICKY M. Biochar-Assisted Phytostabilization for Potentially Toxic Element Immobilization. Sustainability, **14** (1), 445, **2022**.
67. MALAYERI B.E., CHEHREGANI A., YOUSEFI N., LORESTANI B. Identification of the hyper accumulator plants in copper and iron mine in Iran. Pakistan Journal of Biological Sciences, **11** (3), 490, **2008**.
68. ALRAWASHDEH K.A.B., GUL E., YANG Q., YANG H., BARTOCCI P., FANTOZZI F. Effect of heavy metals in the performance of anaerobic digestion of olive mill waste. Processes, **8** (9), 1146, **2020**.
69. KADAM R., KHANTHONG K., JANG H., LEE J., PARK J. Occurrence, Fate, and Implications of Heavy Metals during Anaerobic Digestion: A Review. Energies, **15** (22), 8618, **2022**.
70. KINTL A., VÍTĚZ T., HUŇADY I., BRTNICKÝ M., NOVÁK D., LOCHMAN J., VÍTĚZOVÁ M., HAMMERSCHMIEDT T., HOLÁTKO J., ELBL J. Anaerobic Fermentation of Silage from the Above-Ground Biomass of Jerusalem Artichoke (*Helianthus tuberosus* L.) and Maize (*Zea mays* L.) as a New and Promising Input Raw Material for Biogas Production. Applied Sciences, **12** (22), 11417, **2022**.
71. LI Y., ZHANG R., LIU G., CHEN C., HE Y., LIU X. Comparison of methane production potential, biodegradability, and kinetics of different organic substrates. Bioresources and Technology, **149**, 565, **2013**.
72. KINTL A., ELBL J., VÍTĚZ T., BRTNICKÝ M., SKLÁDANKA J., HAMMERSCHMIEDT T., VÍTĚZOVÁ M. Possibilities of Using White Sweetclover Grown in Mixture with Maize for Biomethane Production. Agronomy, **10** (9), 1407, **2020**.
73. RENGGAMAN A., CHOI H.L., SUDIARTO S.I.A., FEBRISIANTOSA A., AHN D.H., CHOUNG Y.W., SURESH A. Biochemical methane potential of swine slaughter waste, swine slurry, and its codigestion effect. Energies, **14** (21), 7103, **2021**.
74. AMON T., AMON B., KRYVORUCHKO V., MACHMÜLLER A., HOPFNER-SIXT K., BODIROZA V., HRBEK R., FRIEDEL J., PÖTSCH E., WAGENTRISTL H., SCHREINER M., ZOLLITSCH W. Methane production through anaerobic digestion of various energy crops grown in sustainable crop rotations. Bioresources Technology, **98** (17), 3204, **2007**.
75. HERRMANN C., PLOGSTIES V., WILLMS M., HENGELHAUPT F., EBERL V., ECKNER J., STRAUß C., IDLER C., HEIERMANN M. Methane production potential of various crop species grown in energy crop rotations. Landtechnik, **71** (6), 194, **2016**.
76. GUNASEELAN V.N. Biochemical methane potential of fruits and vegetable solid waste feedstocks. Biomass and Bioenergy, **26** (4), 389, **2004**.
77. AMON T., KRYVORUCHKO V., AMON B., MOITZI G., LYSON D., HACKL E., JEREMIC D., ZOL-LITSCH W., PÖTSCH E. Optimierung der Biogaserzeugung aus den Energie-pflanzen Mais und Kleegras, Forschungsprojekt Nr.1249, 1st ed.; Institut für Land Umwelt- und Energietechnik, BOKU, Wien, Austria, pp. 1, **2003** [In German].
78. PAKARINEN O., LEHTOMÄKI A., RISSANEN S., RINTALA J. Storing energy crops for methane production: Effects of solids content and biological additive. Bioresource Technology, **99** (15), 7074, **2008**.
79. KAISER F. Methanerträge verschiedener nachwachsender Rohstoffe. Biogas Journal, **2** (22), **2004** [In German].
80. FERDEȘ M., PARASCHIV G., IONESCU M., DINCĂ M.N., MOICEANU G., ZĂBAVĂ B.Ș. Anaerobic co-digestion: A way to potentiate the synergistic effect of multiple substrates and microbial diversity. Energies, **16** (5), 2116, **2023**.
81. LEHTOMÄKI A., VIINIKAINEN T.A., RINTALA J.A. Screening boreal energy crops and crop residues for methane biofuel production. Biomass and Bioenergy, **32** (6), 541, **2008**.
82. HEIERMANN M., PLÖCHL M., LINKE B., SCHELLE H., HERRMANN C. Biogas Crops - Part 1: Specifications and suitability of field crops for anaerobic digestion. Agricultural Engineering International: CIGR Journal, **11**, 1, **2009**. **.**
83. ZHANG W., KONG T., XING W., LI R., YANG T., YAO N., LV D. Links between carbon/nitrogen ratio, synergy and microbial characteristics of long-term semi-continuous anaerobic codigestion of food waste, cattle manure and corn straw. Bioresource Technology, **343**, 126094, **2021**.

ANNEX

Table A1. Contents of carbohydrates, proteins, lipids and theoretical methane yield (TMY) in biomass obtained from the maize monoculture (M) and from the mixed cropping system (MCS) composed of maize (M-MCS) and bean (B-MCS).

Variant	Year	Carbohydrates (%)		Proteins (%)		Lipids (%)		TMY	
		mean	±SD	mean	±SD	mean	±SD	mean	±SD
M	2020	76.13	3.36	9.48	1.08	1.90	0.19	0.386	0.015
M-MCS		79.83	2.01	10.29	1.07	2.02	0.22	0.397	0.013
B-MCS		48.75	8.84	20.48	2.36	1.63	0.35	0.320	0.027
M	2021	75.18	3.35	6.51	0.20	2.90	0.66	0.375	0.017
M-MCS		83.23	7.02	6.12	0.72	2.07	0.65	0.399	0.034
B-MCS		61.5	2.34	15.89	0.84	1.75	0.47	0.352	0.014

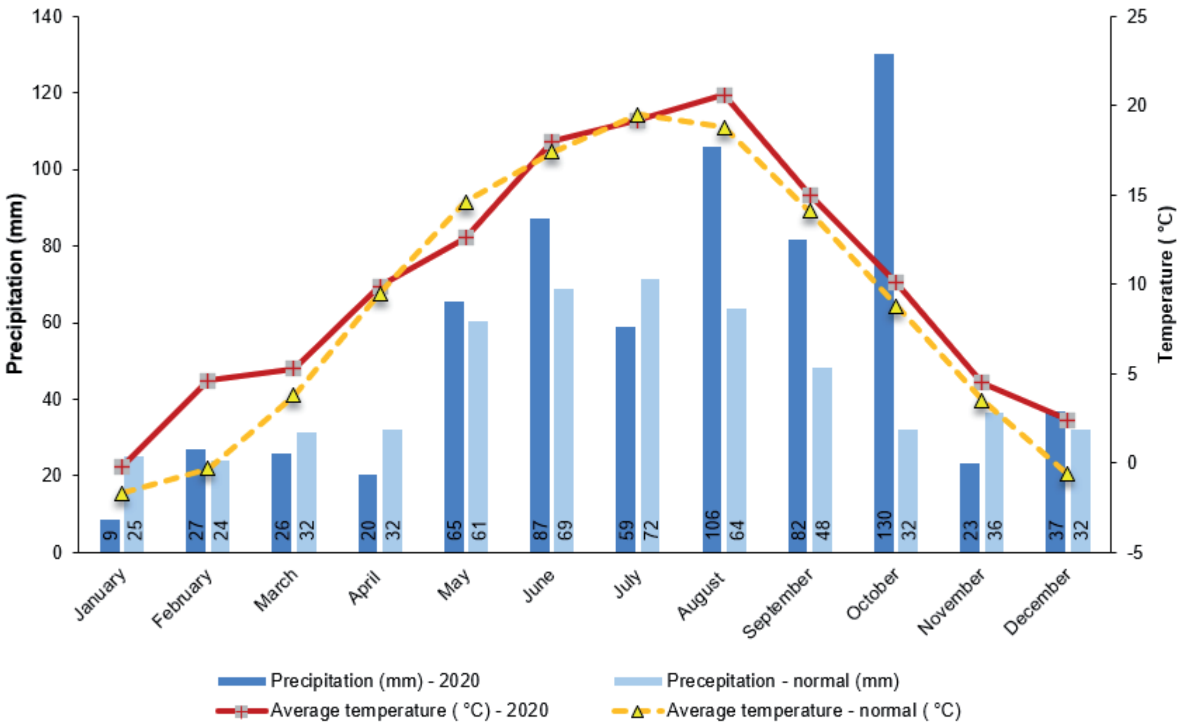

Fig. A1. Precipitation amounts (mm) and temperatures (°C) in 2020 and mean precipitation and temperature values from 1981 to 2020 (Troubsko experimental site).

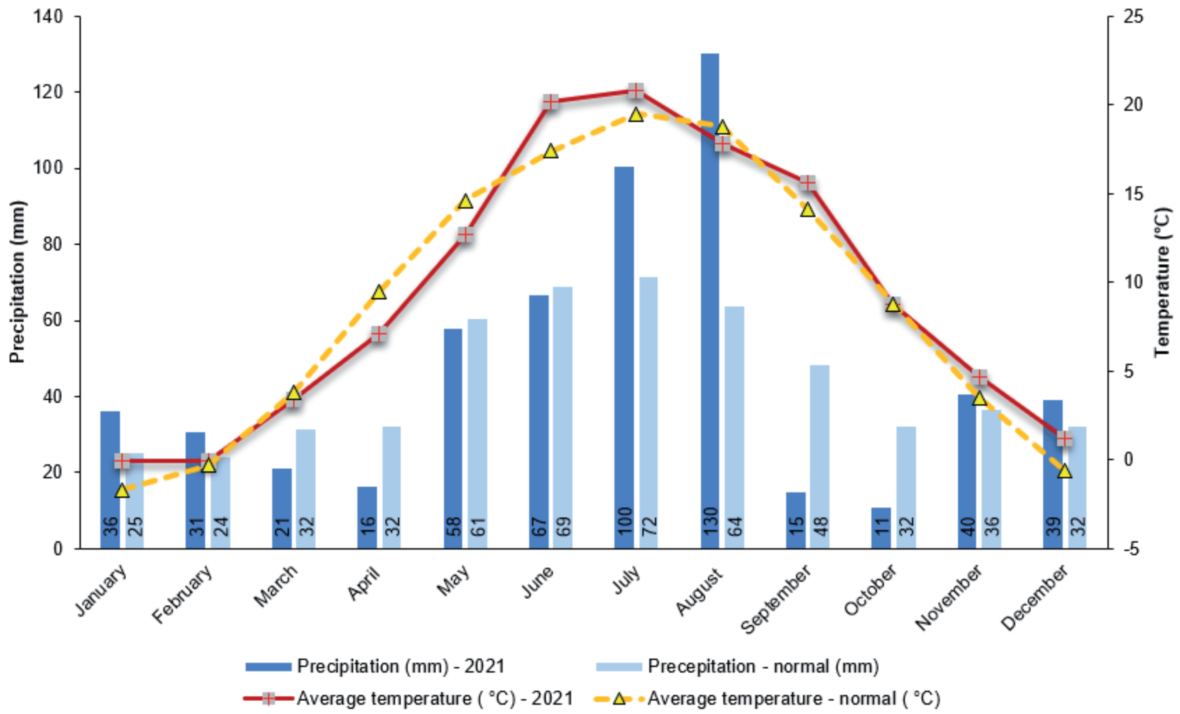

Fig. A2. Precipitation amounts (mm) and temperatures (°C) in 2021 and mean precipitation and temperature values from 1981 to 2020 (Troubsko experimental site).

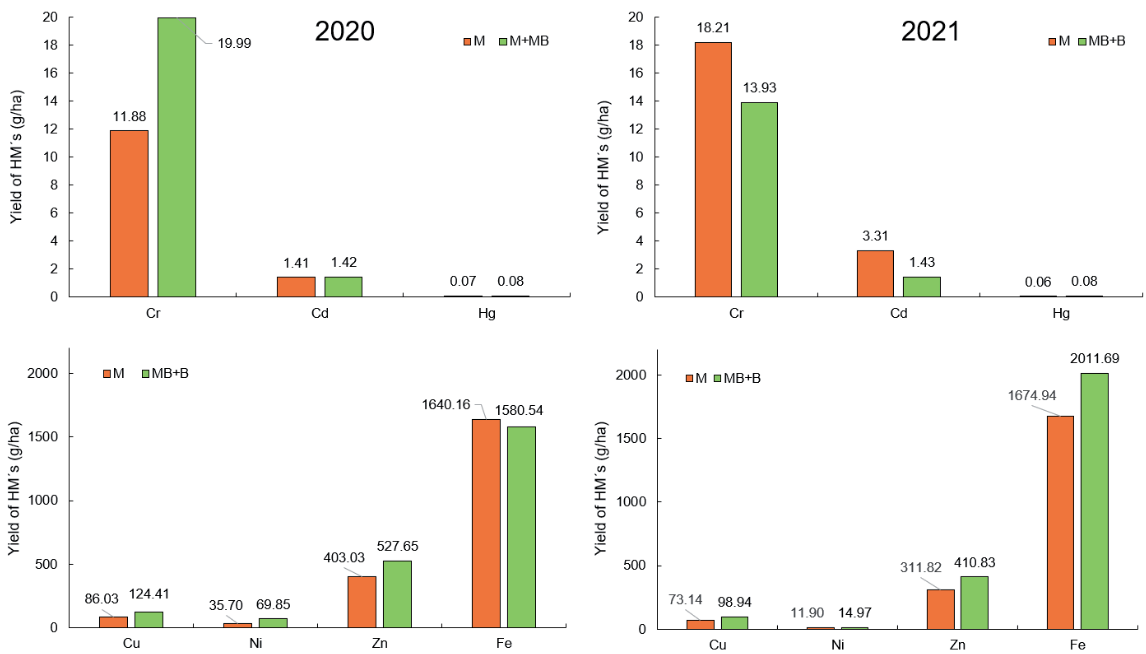

Fig. A3. Cumulative presence of heavy metals in the maize monoculture (M) and in the mixed cropping system (MCS) composed of maize and bean (M-MCS+B-MCS), calculated from the mean yield of crops (t/ha) and the mean concentrations of heavy metals in the crop biomass in 2020 and 2021.