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Digestate and Manure Use in Kohlrabi Production: Impact on Plant-Available Nutrients and Heavy Metals in Soil, Yield, and Mineral Composition

Dragan Kovačević [1,*], Maja Manojlović [1], Ranko Čabilovski [1], Zoran S. Ilić [2], Klara Petković [1], Mirna Štrbac [1] and Mirjana Vijuk [1]

[1] Faculty of Agriculture, University of Novi Sad, 8 Trg Dositeja Obradovića, 21000 Novi Sad, Serbia; maja.manojlovic@polj.uns.ac.rs (M.M.); ranko.cabilovski@polj.uns.ac.rs (R.Č.); klara.petkovic@polj.uns.ac.rs (K.P.); mirna.strbac@polj.uns.ac.rs (M.Š.); mirjana.trninic@polj.uns.ac.rs (M.V.)

[2] Faculty of Agriculture, University of Priština-Kosovska Mitrovica, Kopaonička bb, 38219 Lešak, Serbia; zoran.ilic63@gmail.com

* Correspondence: dragan.kovacevic@polj.uns.ac.rs

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Abstract: Digestate is a residue of the anaerobic decomposition of organic waste for biogas extraction, but it can be reused as a source of nutrients. To examine the effect of digestate in kohlrabi production, field experiments were conducted during three seasons in two calendar years. The fertilization treatments included the application of solid digestate (two rates—DS1 and DS2), liquid digestate (two rates—DL1 and DL2), solid manure (two rates—MS1 and MS2), and mineral fertilizer (NPK) and were compared with a plot without fertilization (Ø). The results showed a significant increase in the yield with the use of solid and liquid digestate, as well as with NPK, in all growing seasons, while the microelement contents (Zn, Mn, and Cu) in the leaves were at optimum level. The applied treatments did not increase the plant-available nutrients (AL-P_2O_5, AL-K_2O, Fe, Cu, and Zn) in the soil (except Mn). The application of DL2, MS1, and MS2 led to a higher Pb content in kohlrabi stems compared to the control, but the Pb content remained below the maximum permitted limit. Our research showed that digestate can be used as a valuable source of nutrients for kohlrabi production, with a low risk of soil and plant contamination by heavy metals. However, the control of soil, digestates, and manure quality is recommended.

Keywords: kohlrabi quality; organic fertilizers; solid; liquid; digestate

1. Introduction

The increase in the world population would not be possible without a parallel growth in food production that can be achieved thanks to the use of fertilizers [1]. The intensive use of high doses of mineral fertilizers, especially nitrogen (N) ones, has led to several issues: a high cost, possible nitrate pollution, and the loss of soil carbon (C). Given this, the need for environmentally friendly agricultural production is growing, and therefore organic amendments such as composts or manures have been extensively studied in the past. Additionally, in recent years, the possibility of using digestate as organic amendments for improving soils has been investigated [2–4]. Digestate can also be used as a fertilizer, allowing the circulation of plant nutrients while reducing the need for mineral fertilizers [5]. Reducing the use of mineral fertilizers leads to positive effects concerning resource conservation, climate change mitigation, and soil quality maintenance [2].

Digestate is formed as a result of the anaerobic digestion process, i.e., the process of fermentation, one of the methods that is widely known for organic waste utilization. The result of this process is biogas and postdigestion matter, a nutrient-rich plant residue (digestate), which can be used as a fertilizer in agriculture [3]. Digestate may contain significant amounts of nutrients such as N, phosphorus (P), and potassium (K) in an easily available form for plants, and because of the rapidity of the release of nutrients from digestate, it resembles mineral fertilizers. It also contains a portion of organic matter, which can positively affect the physicochemical properties of soils [6,7]. According to some authors [8,9], solid digestates show a greater mineral N fraction (51–68% of total N), but other authors [10] found that some solid digestates have a lower mineral N fraction (24–36% of total N). As a soil amendment, the solid fraction of digestate has better potential than the liquid form, while the liquid fraction has better potential as a fertilizer than the solid form. However, the potential contamination of waters (surface and ground) with excess N and P by digestate soil application is a major environmental concern [11]. Additionally, much like some fertilizers (e.g., raw phosphates, industrial compost, and sewage sludge), digestate may contain heavy metals or different organic pollutants [6,12]. To prevent the accumulation of heavy metals, reducing their input into the soil is a strategic goal of soil protection policies in the Republic of Serbia [13]. According to Manojlovic et al. [14], before application in agriculture, it is necessary to analyze the composition of fertilizers and respect the maximum permissible concentrations of heavy metals in fertilizers, soil improvers, and special products, regulating their input into soils [13].

The use of digestate as a fertilizer has been studied in the production of different crops: watermelon [2], kohlrabi [15], alfalfa and spring wheat cultivation [16], tomato [17], etc. Kohlrabi (*Brassica oleracea var. gongylodes*) belongs to the family *Brassicaceae* and is a vegetable closely related to cabbage, usually grown for its edible stem, but its leaves can also be eaten. It is a fast-growing plant that can be grown during spring, summer, and autumn for market supply, so it can be eaten fresh all year [18]. Because of the juicy texture and crisp and sweet taste of its edible stem, it is greatly valued. According to the USDA [19], 100 g of kohlrabi (fresh matter) contains around 1.7 g proteins, 6.2 g carbohydrates, and 3.6 g dietary fiber. It also contains different amounts of vitamins, such as vitamin C, vitamin B6, folate, niacin, thiamin, riboflavin, and pantothenic acid [20].

The research on digestates has mainly been dedicated to stability estimation to reduce odor, decay, and pathogens [11]. To the extent of our knowledge, the impacts of biogas digestate applications on the environment in terms of the availability of nutrients and heavy metals in soil have still not been explored enough. Following the stated facts, the objectives of this research were to examine the impact of digestate, manure, and mineral fertilizers on: (i) plant nutrient availability in soil, (ii) heavy metal availability in soil, and (iii) kohlrabi yield and mineral composition.

2. Materials and Methods

2.1. Experimental Site Description

The experiment was conducted for three growing seasons during the period 2019–2020 on three fields (to respect the crop rotation) that were used for vegetable production. The first growing season (autumn 2019) was at the Futog site, while the second (spring 2020) and third (autumn 2020) seasons were at the Nemanovci site. Both sites are located in the vicinity of Novi Sad, Serbia (Futog—45°15′59.4′′ N, 19°41′09.3′′ E; Nemanovci—45°19′03.8′′ N, 19°51′44.6′′ E and 45°19′05.5′′ N, 19°51′39.8′′ E). On both sites, the soil type is Luvic Chernozem [21], one of the most dominant soil types in the Vojvodina province. Some of the main features that characterize this soil type are the neutral to slightly alkaline reaction of the soil, as well as the good supply of easily available AL-P_2O_5 (ammonium lactate P_2O_5) and AL-K_2O (ammonium lactate K_2O). All three soils fell into the same category regarding pH value (slightly alkaline) and available phosphorus and potassium (optimally provided). The basic physical and chemical properties of the soil are given in Table 1.

Table 1. Basic physical and chemical properties of the soil before conducting the experiment.

Parameter	Season I Autumn 2019 Futog	Season II Spring 2020 Nemanovci	Season III Autumn 2020 Nemanovci
% Coarse sand	0.6	0.1	0.1

Table 1. *Cont.*

Parameter	Season I Autumn 2019 Futog	Season II Spring 2020 Nemanovci	Season III Autumn 2020 Nemanovci
% Fine sand	50.16	43.74	38.98
% Silt	29.84	37.04	37.8
% Clay	19.4	19.12	23.12
pH KCl	7.16 ± 0.07	7.42 ± 0.04	7.21 ± 0.10
pH H_2O	7.83 ± 0.05	8.07 ± 0.04	7.78 ± 0.09
% $CaCO_3$	2.95 ± 0.06	6.26 ± 0.04	4.17 ±0.02
AL-P_2O_5 mg kg^{-1}	175 ± 11.63	302 ± 17.14	194 ± 15.48
AL-K_2O mg kg^{-1}	211 ± 23.65	339 ± 41.47	369 ± 14.94
Available Fe mg kg^{-1}	9.27 ± 1.00	7.97 ± 1.70	11.17 ± 1.53
Available Mn mg kg^{-1}	17.84 ± 1.20	19.21 ± 1.59	18.46 ± 0.81
Available Cu mg kg^{-1}	2.89 ± 0.65	4.62 ± 0.53	5.58 ± 0.71
Available Zn mg kg^{-1}	0.89 ± 0.10	2.96 ± 0.16	3.53 ± 0.44
Available Pb mg kg^{-1}	1.90 ± 0.15	2.42 ± 0.17	2.54 ± 0.08
Available Cr mg kg^{-1}	0.15 ± 0.01	0.04 ± 0.01	0.01 ± 0.00
Available Cd mg kg^{-1}	0.06 ± 0.01	0.08 ± 0.01	0.08 ± 0.01

±, standard error.

The experiment was performed in conditions of drip irrigation. The average air temperatures during the kohlrabi vegetation in 2019 and 2020 were similar to the long-term average (Figure 1a). However, the amount of precipitation in autumn 2019 (Futog) and spring 2020 (Nemanovci) during the kohlrabi vegetation was lower than the long-term average (Figure 1b). On the other hand, the amount of precipitation during the kohlrabi vegetation in autumn 2020 at the Nemanovci site was higher than the long-term average. The mean monthly temperatures and total monthly precipitation during the experiment are given in Figure 1.

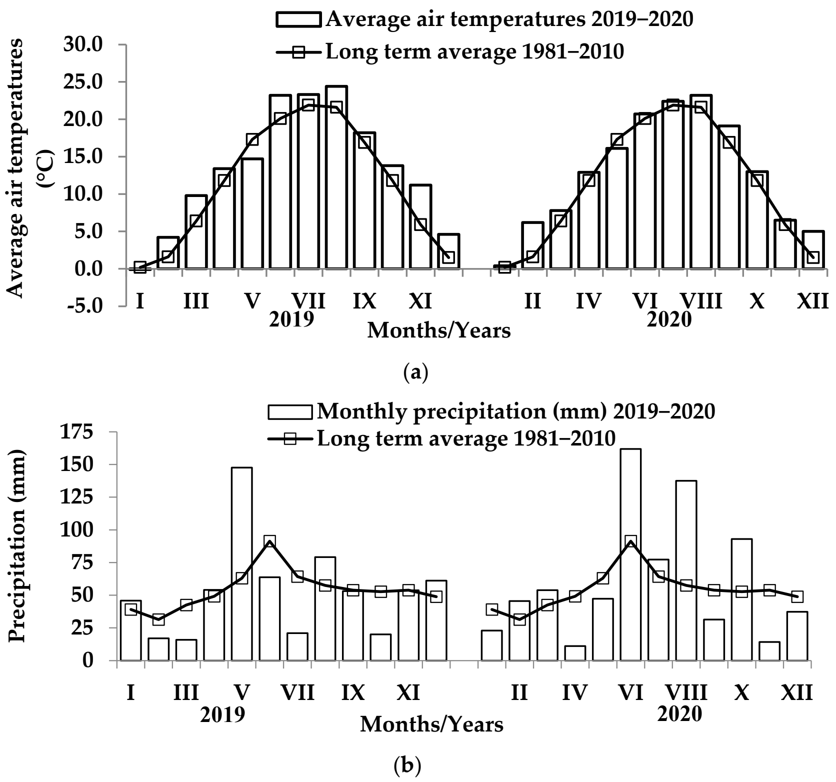


Figure 1. (**a**) Average monthly temperature in Novi Sad (2019−2020) and long-term average (1981−2010); (**b**) monthly precipitation in Novi Sad (2019−2020) and long-term average (1981−2010).

2.2. Experiment Design and Treatments

The experiment was conducted as a completely randomized block system in three replications, and individual plots were 1.75 m long and 1.20 m wide, while the space between the plots was 0.5 m. Each plot consisted of 32 kohlrabi plants (*Brassica oleracea var. gongylodes*), Quickstar (F1) cultivar, Sakata, planted in four rows (8 plants per row). Kohlrabi was planted with 30 cm spacing between the rows and 25 cm spacing within the rows. Eight fertilization treatments were incorporated into the soil:

1. Control treatment—without fertilization (Ø).
2. Solid digestate—100 kg N ha^{-1} (DS1).
3. Solid digestate—200 kg N ha^{-1} (DS2).
4. Liquid digestate—100 kg N ha^{-1} (DL1).
5. Liquid digestate—200 kg N ha^{-1} (DL2).
6. Solid manure—100 kg N ha^{-1} (MS1).
7. Solid manure—200 kg N ha^{-1} (MS2).
8. Standard fertilization with mineral fertilizers (NPK).

In total, there were 24 experimental plots for each season. Organic fertilizers were applied in the amount which brought 100 kg N ha^{-1} and 200 kg N ha^{-1} to soil (depending on the treatments), while mineral fertilizers (treatment 8) were applied in the amount of 100 kg N ha^{-1}, 80 kg P_2O_5, and 100 kg K_2O, as ammonium nitrate, superphosphate, and potassium chloride, respectively. This meant that solid digestate was applied in the amounts of 20.8 t ha^{-1} (DS1) and 41.7 t ha^{-1} (DS2), liquid digestate in the amounts of 16.4 t ha^{-1} (DL1) and 32.8 t ha^{-1} (DL2), and manure in the amounts of 14.1 t ha^{-1} (MS1) and 28.2 t ha^{-1} (MS2). The chemical compositions of applied fertilizers are presented in Table 2. The heavy element content in all fertilizers was below the French regulation by ministerial decree [22].

Table 2. Chemical properties of organic fertilizers and annual inputs of elements.

Chemical Properties of Fertilizers				Regulation		Annual Inputs of Dry Matter and Elements with the Amount of Fertilizer Equal to 100 kg N ha^{-1}		
Parameter	Solid Digestate	Liquid Digestate	Solid Manure	Heavy Metal Limits **	Heavy Metal Inputs ***	Solid Digestate	Liquid Digestate	Solid Manure
pH H_2O	8.48 ± 0.02	7.48 ± 0.14	8.82 ± 0.07	/	/	/	/	/
C/N	25.6 ± 0.98	4.3 ± 0.05	20.2 ± 0.60	/	/	/	/	/
		% in FM *				t ha^{-1}	t ha^{-1}	t ha^{-1}
DM	28.85 ± 0.78	6.98 ± 0.37	37.56 ± 0.69	/	/	1.75	1.14	1.98
	g kg^{-1} DM *	g kg^{-1} FM	g kg^{-1} DM	/	/		kg ha^{-1}	
OM	618 ± 12.50	50 ± 1.17	714 ± 8.19	/	/	3745	817	3755
Total N	17 ± 0.48	6.1 ± 0.17	19 ± 0.55	/	/	100	100	100
Total C	422 ± 5.89	26 ± 0.59	383 ± 1.43	/	/	2559	430	2018
Total P_2O_5	9.4 ± 0.61	2.2 ± 0.09	9.5 ± 0.29	/	/	57	36	50
Total K_2O	12.8 ± 0.34	3.6 ± 0.10	20.1 ± 0.72	/	/	78	59	106
Mg	4.02 ± 0.04	1.28 ± 0.04	10.37 ± 0.32	/	/	24	21	55
Ca	4.29 ± 0.07	4.14 ± 0.05	7.74 ± 0.07	/	/	26	68	41
	mg kg^{-1} DM	mg kg^{-1} FM	mg kg^{-1} DM	mg kg^{-1} DM	g ha year^{-1}		g ha^{-1}	
Fe	400 ± 8.33	264 ± 9.06	734 ± 27.47	/	/	2424	4334	3862
Mn	65 ± 3.08	32 ± 2.13	102 ± 6.82	/	/	391	524	536
Cu	9.3 ± 0.17	5.2 ± 0.26	15.5 ± 1.01	<600	350	56	85	82
Zn	32.8 ± 0.69	21.5 ± 0.55	58.8 ± 1.22	<1500	1500	199	352	310
Pb	nd	nd	9.04 ± 0.05	<180	300	nd	nd	48
Cr	16.4 ± 0.71	12.2 ± 0.85	19.2 ± 0.35	<120	300	99	200	101
Cd	nd	nd	nd	<3	5	nd	nd	nd

* DM, dry matter; FM, fresh matter; nd, not detected; ±, standard error. ** Maximum heavy metal limits according to the French regulation [22]. *** Regulating inputs of heavy metals to soils (g ha year^{-1}) according to the Serbian regulation [13].

For all treatments, fertilizers were incorporated into the surface soil layer (0–30 cm) 10 days before kohlrabi planting. Kohlrabi seedlings were prepared by sowing seeds in 104 styrofoam seedling tray cavities (Futog 12/7/2019; Nemanovci 28/2/2020 and 20/7/2020) filled with a substrate for vegetable seedlings. Kohlrabi plants were transplanted after 36 days in autumn 2019, after 38 days in spring 2020, and after 39 days in autumn 2020, using a manual vegetable seedling transplanter. After transplanting the plants, a drip irrigation system was installed between the rows. At the time of transplanting, kohlrabi was watered with around 30 mm of water, and then, every 5–8 days until the thickening of the stem, with around 20 mm of water.

2.3. Sampling

2.3.1. Soil

Soil samples for analyzing the basic soil properties (Table 1) were taken every season before setting up the experiment. On every experimental site, three average samples (composed of 10 individual samples) were taken from the 0–30 soil layer. Additionally, during the experiment, soil samples were taken twice from every treatment repetition ($8 \times 3 = 24$ experimental plots). The first sampling (24 composite soil samples) was carried out 10 days after fertilizer incorporation (before planting kohlrabi), and the second (24 composite soil samples) after harvesting kohlrabi. The one composite sample which represents an experimental plot was consistent with 5 individual soil samples collected from the same treatment repetition (0–30 cm depth). Samples were collected with a soil probe. There were 48 soil samples per season, and so there were 144 soil samples in total, plus an additional 9 samples for the analysis before the experimental set up. In the laboratory, the soil samples were air-dried, ground, and sieved through a 2 mm sieve.

2.3.2. Digestate and Manure

Digestates (solid and liquid) and manure used in all growing seasons were collected from the biogas plant Stara Moravica (Serbia). The basic feedstock for biogas production was chopped cattle manure, maize silage, and haylage. The solid digestate used in the experiment was taken fresh from the pile, while the liquid digestate was also taken fresh from the lagoon. The manure used as feedstock for biogas production was applied in the experiment.

2.3.3. Kohlrabi

Kohlrabi was harvested when it was commercially ripened. During the harvest, the mass of 4 enlarged overground kohlrabi stems and leaves were measured from two middle rows (8 plants in total) on each plot to determine the total yield and the content of macro and microelements, as well as heavy metal content. The total enlarged kohlrabi stem and leaf yield (g m^{-2}) was calculated on the basis of the mean weight of 8 separate enlarged kohlrabi stems and leaves multiplied by the number of kohlrabi per square meter.

2.4. Analytical Determination

Analytical determination was performed on the samples of soil, digestate, manure, and enlarged kohlrabi stems and leaves.

2.4.1. Soil Analysis

Available P (AL-P_2O_5) and K (AL-K_2O) were extracted from the soil with an AL solution of 0.1 mol L^{-1} ammonium lactate and 0.4 mol L^{-1} acetic acid, in the ratio of 1:20 (soil:solution) [23]. The available P_2O_5 content was determined spectrophotometrically (Shimadzu, UV-2600, Kyoto, Japan), and the K_2O content by flame photometry (Jenway 6105, Essex, UK). Mineral N concentration in the soil was determined by the Bremner [24] method. The available iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), lead (Pb), chromium (Cr), and cadmium (Cd) concentrations in the soil were determined after the extraction with DTPA (diethylenetriaminepentaacetic acid)–TEA (triethanolamine) (0.005 mol L^{-1} DTPA + 0.01 mol L^{-1} $CaCl_2$ + 0.1 mol L^{-1} TEA) buffered solution in the ratio 1:2 (soil:solution) [25]. The concentration was measured using an atomic absorption spectrometer (AAS, Shimadzu 6300, Kyoto, Japan) with the flame (FAAS) (Fe, Mn, Cu, Zn) and graphite (GFAAS) (Pb, Cr, Cd) techniques.

2.4.2. Digestate and Manure Analysis

The pH value of fresh solid samples was determined in water suspension, in the ratio of 1:5, using a Metrel Toledo FE 20 pH meter, while the pH of liquid samples was determined without water addition [26]. The dry matter content of fertilizers was determined gravimetrically after drying the samples to constant weight (70 °C for 24 h). Organic matter content was determined after incineration (loss on ignition) in an oven after 5 h at 550 °C [27]. The total C, N, and C/N contents were determined using a CHNS analyzer (Elementar Vario EL, GmbH, Hanau, Germany). After the microwave-assisted digestion of organic fertilizers with concentrated HNO_3 acid [28], the flame technique (FAAS) was used to determine the contents of magnesium (Mg), calcium (Ca), Fe, Mn, Cu, and Zn, and the graphite technique (GFAAS) was used for Pb, Cr, and Cd determination. The phosphorus content (P_2O_5) was determined in the extract via molybdenum blue method [29] spectrophotometrically (Shimadzu, UV-2600, Kyoto, Japan), and the K_2O content by flame photometry (Jenway 6105, Essex, UK).

2.4.3. Kohlrabi Bulb and Leaf Analyses

In the laboratory, the kohlrabi samples were washed several times with distilled water, and the excess water was wiped off using filter paper. Bulb slices and leaves separately obtained from 8 whole kohlrabies from each treatment were homogenized in a blender to obtain a representative average sample. After homogenization, fresh samples were dried in a food dryer (Excalibur) for 24 h at the temperature of 52 °C degrees. The average dry matter content of enlarged stems and leaves was calculated based on the dry matter content of 10 separate enlarged kohlrabi stems and leaves. Analyses were performed on dry samples. To determine the total Fe, Mn, Cu, Zn, Pb, Cr, and Cd content in enlarged kohlrabi stems and leaves, wet digestion was used, with a mixture of nitric (HNO_3) and perchloric ($HClO_4$) acid [30], and the measuring was performed by AAS.

2.5. Statistics

The results from the study were subjected to a two-way analysis of variance (ANOVA), whereas the LSD test ($p < 0.05$) was used to detect the significant differences between the measured variables among treatments in STATISTICA 14 software. The first factor was the fertilization treatments, and the second factor was the growing season.

3. Results

3.1. Plant-Available Nutrients

The plant-available nutrient concentrations in the soil over three growing seasons, 10 days after fertilizer application (a day before planting) and after the kohlrabi harvest, are shown in Table 3. The fertilization treatments did not have a significant influence on the plant-available P_2O_5 and K_2O, Mn, Cu, and Zn 10 days after fertilizer application. The plant-available Fe (DTPA-Fe) in the soil was reduced significantly with all fertilization treatments (except the NPK treatment) compared to the unfertilized control (10 days after fertilizer application). The growing season was a significant factor affecting the nutrient concentration in the soil. This is also related to there being different nutrient concentrations in the different fields where the experiments were set up.

After the kohlrabi harvest, the fertilization treatments did not have a significant influence on the plant-available P_2O_5 and K_2O, Cu, and Zn. The organic fertilizer had neither a positive nor a negative effect on the DTPA-Fe (after kohlrabi harvest). However, the fertilization treatments, as well as the growing season, significantly affected the Mn concentration in the soil. The plant-available Mn (DTPA-Mn) increased significantly with the application of all treatments compared to the unfertilized control. Additionally, the treatment with DL2 increased the DTPA-Mn concentration in the soil significantly compared to the DS1 and DS2 treatments. The growing season was also a significant factor affecting the nutrient concentration in the soil after harvest.

Table 3. Plant-available nutrients in the soil (mg kg^{-1}) as a result of digestates, manure, and mineral fertilizer application.

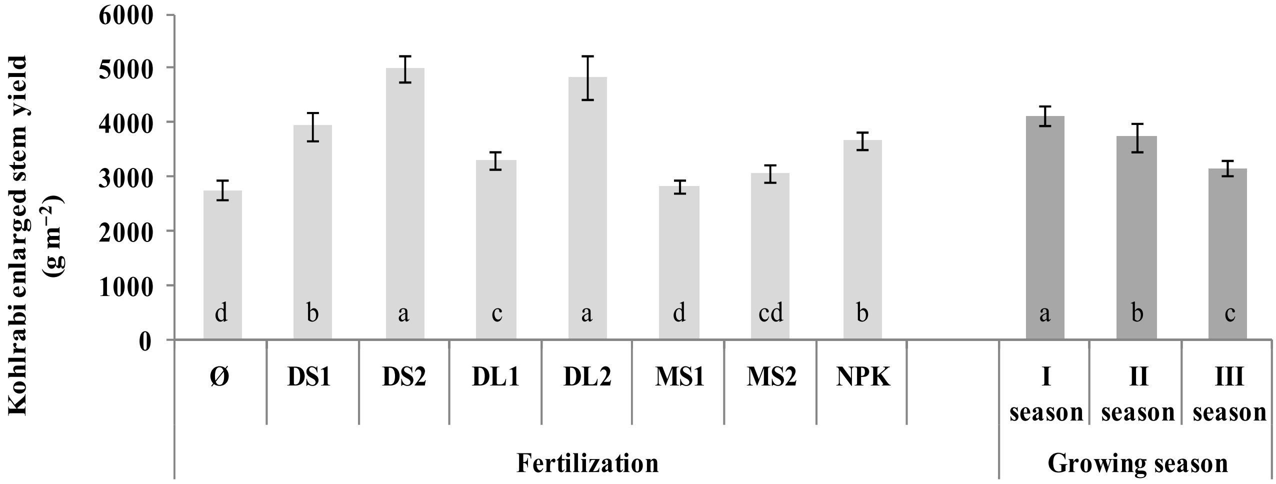
Treatment	10 Days after Fertilizer Application						after Kohlrabi Harvest					
	AL-P_2O_5	AL-K_2O	Fe	Mn	Cu	Zn	AL-P_2O_5	AL-K_2O	Fe	Mn	Cu	Zn
Ø	219 ^{a}	314 ^{a}	11.9 ^{a}	17.3 ^{ab}	4.6 ^{ab}	2.5 ^{ab}	230 ^{ab}	337 ^{b}	9.5 ^{a}	9.6 ^{c}	5.5 ^{a}	2.3 ^{a}
DS1	214 ^{a}	337 ^{a}	10.4 ^{bc}	17.8 ^{ab}	5.2 ^{ab}	3.2 ^{a}	240 ^{ab}	375 ^{a}	11.0 ^{a}	13.3 ^{b}	5.1 ^{ab}	2.1 ^{a}
DS2	227 ^{a}	340 ^{a}	10.1 ^{bc}	17.6 ^{ab}	4.2 ^{ab}	2.5 ^{ab}	240 ^{ab}	363 ^{ab}	10.8 ^{a}	13.2 ^{b}	5.0 ^{ab}	2.2 ^{a}
DL1	206 ^{a}	322 ^{a}	9.5 ^{cd}	17.4 ^{ab}	3.9 ^{b}	2.3 ^{b}	210 ^{b}	357 ^{ab}	10.2 ^{a}	13.6 ^{ab}	5.0 ^{ab}	2.3 ^{a}
DL2	217 ^{a}	319 ^{a}	9.8 ^{bcd}	17.9 ^{ab}	4.3 ^{ab}	2.4 ^{ab}	240 ^{ab}	365 ^{ab}	9.7 ^{a}	14.8 ^{a}	4.8 ^{ab}	2.1 ^{a}
MS1	210 ^{a}	348 ^{a}	8.6 ^{d}	18.5 ^{a}	4.5 ^{ab}	2.5 ^{ab}	220 ^{ab}	360 ^{ab}	10.2 ^{a}	13.5 ^{ab}	4.7 ^{b}	2.0 ^{a}
MS2	220 ^{a}	346 ^{a}	8.5 ^{d}	18.2 ^{a}	4.3 ^{ab}	2.6 ^{ab}	250 ^{a}	357 ^{ab}	9.8 ^{a}	13.8 ^{ab}	4.9 ^{ab}	2.2 ^{a}
NPK	242 ^{a}	326 ^{a}	10.8 ^{ab}	16.2 ^{b}	5.3 ^{a}	3.0 ^{ab}	250 ^{a}	350 ^{ab}	9.7 ^{a}	13.4 ^{ab}	5.3 ^{ab}	2.2 ^{a}
Growing season												
Season I	153 ^{c}	205 ^{c}	14.5 ^{a}	13.9 ^{c}	3.2 ^{c}	1.1 ^{b}	149 ^{c}	239 ^{b}	13.3 ^{a}	9.1 ^{c}	4.0 ^{c}	1.0 ^{c}
Season II	318 ^{a}	377 ^{b}	7.1 ^{c}	20.4 ^{a}	4.8 ^{b}	3.3 ^{a}	303 ^{a}	415 ^{a}	7.5 ^{c}	19.4 ^{a}	6.0 ^{a}	2.5 ^{b}
Season III	188 ^{b}	412 ^{a}	8.3 ^{b}	18.5 ^{b}	5.6 ^{a}	3.5 ^{a}	251 ^{b}	420 ^{a}	9.6 ^{b}	11.0 ^{b}	5.1 ^{b}	3.1 ^{a}
Source of variation												
T	ns	ns	*	ns	ns	ns	ns	ns	ns	*	ns	ns
G	*	*	*	*	*	*	*	*	*	*	*	*
TxG	ns	ns	*	ns	ns	ns	ns	ns	*	*	ns	ns

Ø, control; DS1, solid digestate 100 kg N ha^{-1}; DS2, solid digestate 200 kg N ha^{-1}; DL1, liquid digestate 100 kg N ha^{-1}; DL2, liquid digestate 200 kg N ha^{-1}; MS1, solid manure 100 kg N ha^{-1}; MS2, solid manure 200 kg N ha^{-1}; NPK, mineral fertilizer. Values followed by different letters are significantly different at $p < 0.05$. Source of variation (ns, not significant; *, significant at significance level p <0.05; T, fertilization treatments; G, growing season; TxG, interaction between treatments and growing season).

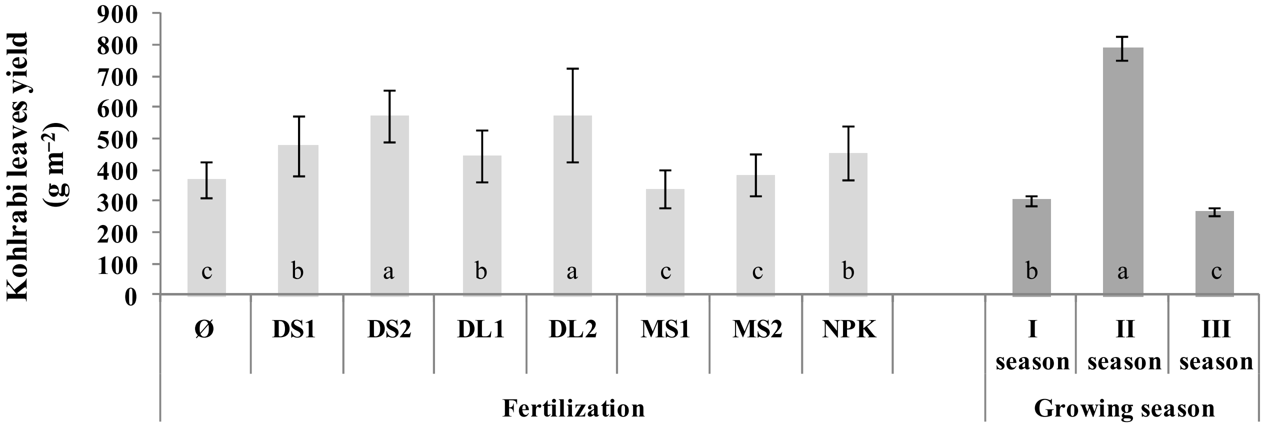
3.2. Total Kohlrabi Yield

The application of solid and liquid digestates (treatments DS1, DS2, DL1, and DL2), as well as the NPK fertilizers, had a significant effect on the total yield of kohlrabi (Figure 2a). The yield of enlarged kohlrabi stems ranged from 3292 to 5005 g m^{-2} for the DS1, DS2, DL1, DL2, and NPK treatments and was significantly higher compared to the yield achieved with the control (2760 g m^{-2}), MS1 (2839 g m^{-2}), and MS2 (3074 g m^{-2}) treatments. The highest kohlrabi bulb yield was achieved with the application of solid digestate in the amount which brought 200 kg N ha^{-1} (DS2) into the soil. The sequence of the increasing kohlrabi yield was as follows: DS2 > DL2 > DS1 > NPK > DL1. The total yield obtained with the MS1 and MS2 treatments was similar to the yield achieved with the control. The growing season was also a significant factor that influenced the kohlrabi bulb yield. The highest yield was obtained in the first growing season (autumn 2019), followed by the second (spring 2020), and then the third (autumn 2020).

The mass of the kohlrabi leaves was significantly affected by the fertilization treatments and growing season (Figure 2b). The highest kohlrabi mass yield was achieved with the application of solid and liquid digestate in the amount which brought 200 kg N ha^{-1} (DS2 and DL2) into the soil. Additionally, a higher leaf mass was achieved with the DS1, DL1, and NPK treatments compared to the unfertilized treatment. The highest mass of leaves was obtained in the second growing season (spring 2020), followed by the first (autumn 2019), and then the third (autumn 2020).



(**a**)



(**b**)

Figure 2. (**a**) The total yield of enlarged kohlrabi stems; (**b**) the total yield of kohlrabi leaves. Ø, control; DS1, solid digestate 100 kg N ha^{-1}; DS2, solid digestate 200 kg N ha^{-1}; DL1, liquid digestate 100 kg N ha^{-1}; DL2, liquid digestate 200 kg N ha^{-1}; MS1, solid manure 100 kg N ha^{-1}; MS2, solid manure 200 kg N ha^{-1}; NPK, mineral fertilizer. Values followed by different letters are significantly different at $p < 0.05$.

3.3. Available (DTPA) Heavy Metals in Experimental Soils

The DTPA-extractable heavy metal levels in the soils are shown in Table 4. The fertilization treatments did not result in a significant increase or decrease in the Pb and Cr concentration in the soil 10 days after fertilizer application, while MS1 and MS2 reduced the Cd concentration compared to control. The growing season was a significant factor affecting the heavy metal concentration in the soil, due to the different plots on which the experiment was performed and the different heavy metal concentrations in the soil.

Table 4. Available heavy metal concentration in soil (mg kg^{-1}) as a result of digestates, manure, and mineral fertilizer application.

Treatment	10 Days after Fertilizer Application			After Kohlrabi Harvest		
	Pb	Cr	Cd	Pb	Cr	Cd
Ø	2.280 ^{a}	0.015 ^{a}	0.069 ^{a}	2.099 ^{b}	0.023 ^{a}	0.068 ^{a}
DS1	2.101 ^{a}	0.007 ^{ab}	0.071 ^{a}	2.299 ^{ab}	0.015 ^{ab}	0.060 ^{ab}
DS2	2.143 ^{a}	0.009 ^{ab}	0.062 ^{ab}	2.313 ^{ab}	0.004 ^{cde}	0.048 ^{c}

Table 4. *Cont.*

Treatment	10 Days after Fertilizer Application			After Kohlrabi Harvest		
	Pb	Cr	Cd	Pb	Cr	Cd
DL1	2.143 [a]	0.008 [ab]	0.067 [ab]	2.122 [b]	0.003 [de]	0.064 [ab]
DL2	2.280 [a]	0.014 [a]	0.067 [ab]	2.255 [ab]	0.013 [bc]	0.067 [ab]
MS1	2.105 [a]	0.013 [a]	0.056 [bc]	2.406 [a]	0.012 [bcd]	0.068 [a]
MS2	2.247 [a]	0.011 [ab]	0.049 [c]	2.407 [a]	0.003 [de]	0.062 [ab]
NPK	2.113 [a]	0.005 [b]	0.061 [ab]	2.403 [a]	0.001 [e]	0.056 [bc]
Growing season						
Season I	1.921 [b]	0.015 [a]	0.044 [c]	2.330 [b]	0.014 [a]	0.058 [b]
Season II	1.919 [b]	0.011 [a]	0.064 [b]	2.014 [c]	0.009 [ab]	0.070 [a]
Season III	2.690 [a]	0.005 [b]	0.080 [a]	2.521 [a]	0.006 [b]	0.056 [b]
Source of variation						
T	ns	ns	*	*	*	*
G	*	*	*	*	*	*
TxG	ns	ns	*	ns	*	*

Ø, control; DS1, solid digestate 100 kg N ha^{-1}; DS2, solid digestate 200 kg N ha^{-1}; DL1, liquid digestate 100 kg N ha^{-1}; DL2, liquid digestate 200 kg N ha^{-1}; MS1, solid manure 100 kg N ha^{-1}; MS2, solid manure 200 kg N ha^{-1}; NPK, mineral fertilizer. Values followed by different letters are significantly different at $p < 0.05$. Source of variation (ns, not significant; *, significant at significance level $p < 0.05$; T, fertilization treatments; G, growing season; TxG, interaction between treatments and growing season).

The fertilization treatment as a factor significantly affected the Pb, Cr, and Cd concentrations in the soil after harvesting the kohlrabi. The application of manure (MS2 and MS1) and NPK fertilizers increased the concentration of Pb in the soil compared to the control and DL1 treatment. On the other hand, the Cr concentration in the soil was reduced with the application of all treatments compared to control, while the Cd concentration decreased with the DS2 and NPK treatments. The growing season was a significant factor for the concentration of heavy metals in the soil. In the second growing season (spring 2020), the Cd concentration in the soil after the harvest was higher compared to the concentration in the first and third growing seasons (autumn 2019 and autumn 2020).

3.4. Mineral Composition of Enlarged Kohlrabi Stems

The mineral composition of enlarged kohlrabi stem in the terms of microelements (Fe, Mn, Cu, and Zn) is shown in Table 5. The effect of the fertilization treatments was not equal for all the examined microelements. Fertilization treatments, as well as growing seasons, were significant factors for the Fe content in the enlarged stem. The content of Fe decreased significantly with DS2, DL2, MS1, MS2, and NPK treatments, compared to control, and DL1 treatment. Additionally, application of liquid digestate (DL2 treatment) decreased Mn and Zn content in the enlarged stem. However, the source of variation for Zn content in enlarged kohlrabi stem was not either the treatments, or growing seasons.

Table 5. Mineral composition (mg kg^{-1}) of enlarged kohlrabi stem (dry matter) as a result of digestates, manure and mineral fertilizer application.

Treatment	Fe	Mn	Cu	Zn
Ø	170 [a]	19.9 [ab]	6.4 [ab]	20.4 [a]
DS1	152 [ab]	18.1 [abc]	6.6 [a]	20.2 [ab]
DS2	127 [bc]	17.8 [abc]	6.2 [ab]	20.0 [abc]
DL1	168 [a]	20.0 [a]	6.3 [ab]	19.8 [abc]
DL2	132 [bc]	16.0 [c]	6.1 [ab]	17.6 [c]
MS1	109 [c]	16.6 [bc]	6.1 [ab]	18.2 [abc]
MS2	102 [c]	19.5 [ab]	6.2 [ab]	19.1 [abc]
NPK	131 [bc]	19.2 [abc]	5.4 [b]	17.7 [bc]

Table 5. *Cont.*

Treatment	Fe	Mn	Cu	Zn
Growing season				
I season	142 ^{b}	13.3 ^{c}	9.5 ^{a}	20.2 ^{a}
II season	98 ^{c}	22.4 ^{a}	4.5 ^{b}	18.9 ^{ab}
III season	169 ^{a}	19.4 ^{b}	4.5 ^{b}	18.3 ^{b}
Source of variation				
T	*	ns	ns	ns
G	*	*	*	ns
TxG	ns	ns	*	ns

Ø, Control; DS1, Digestate solid 100 kg N ha^{-1}; DS2, Digestate solid 200 kg N ha^{-1}; DL1, Digestate liquid 100 kg N ha^{-1}; DL2, Digestate liquid 200 kg N ha^{-1}; MS1, Manure solid 100 kg N ha^{-1}; MS2, Manure solid 200 kg N ha^{-1}; NPK, mineral fertilizer. Values followed by different letters are significantly different at $p < 0.05$. Source of variation (ns, not significant; *, significant at significance level p <0.05; T, fertilization treatments; G, growing season; TxG, interaction between treatments and growing season).

3.5. Mineral Composition of Kohlrabi Leaves

Table 6 shows the mineral composition of kohlrabi leaves (Fe, Mn, Cu, and Zn), which differed significantly among the treatments, depending on the growing season. Fertilization treatments, as well as growing seasons, were significant factors for the Mn content in the leaves. Manganese content in the leaves reduced significantly with the mineral fertilizers application, compared to control. Additionally, the Mn content in the leaves was higher in the second growing season (spring 2020) compared to others. The growing season was the significant source of variation for the mineral composition of kohlrabi leaves.

Table 6. Mineral composition (mg kg^{-1}) of kohlrabi leaves (dry matter) as a result of digestates, manure and mineral fertilizer application.

Treatment	Fe	Mn	Cu	Zn
Ø	735 ^{ab}	86 ^{ab}	9.2 ^{c}	26 ^{ab}
DS1	747 ^{ab}	86 ^{ab}	9.9 ^{bc}	27 ^{ab}
DS2	652 ^{bc}	87 ^{ab}	13.9 ^{a}	34 ^{a}
DL1	733 ^{ab}	83 ^{bc}	11.2 ^{abc}	29 ^{ab}
DL2	854 ^{a}	93 ^{a}	12.3 ^{ab}	29 ^{ab}
MS1	756 ^{ab}	89 ^{ab}	11.3 ^{abc}	30 ^{ab}
MS2	696 ^{abc}	88 ^{ab}	10.3 ^{bc}	26 ^{ab}
NPK	536 ^{c}	75 ^{c}	10.8 ^{abc}	24 ^{b}
Growing season				
I season	1055 ^{a}	69 ^{c}	12.1 ^{b}	39 ^{a}
II season	517 ^{b}	108 ^{a}	15.1 ^{a}	22 ^{b}
III season	568 ^{b}	82 ^{b}	6.2 ^{c}	24 ^{b}
Source of variation				
T	ns	*	ns	ns
G	*	*	*	*
TxG	ns	ns	ns	ns

Ø, Control; DS1, Digestate solid 100 kg N ha^{-1}; DS2, Digestate solid 200 kg N ha^{-1}; DL1, Digestate liquid 100 kg N ha^{-1}; DL2, Digestate liquid 200 kg N ha^{-1}; MS1, Manure solid 100 kg N ha^{-1}; MS2, Manure solid 200 kg N ha^{-1}; NPK, mineral fertilizer. Values followed by different letters are significantly different at $p < 0.05$. Source of variation (ns, not significant; *, significant at significance level p <0.05; T, fertilization treatments; G, growing season; TxG, interaction between treatments and growing season).

3.6. Heavy Metal Content in Enlarged Kohlrabi Stems and Leaves

Table 7 shows the content of heavy metals in the enlarged kohlrabi stems and leaves, which differed significantly among the treatments depending on the growing season. The application of DL2, MS1, and MS2 led to a higher Pb content in the enlarged kohlrabi stems compared to the control and the DS1 treatment. The mineral fertilizers (NPK) also increased the Pb content in the enlarged kohlrabi stems compared to the DS1 treatment. On the other hand, a significantly lower Cd content in the enlarged kohlrabi stems was determined by the application of solid digestate (DS1 and DS2) and manure (MS2) compared to the control, DL1, and DL2 treatments. The lead content in the kohlrabi leaves increased significantly with the NPK treatment compared to control and DS1, DL1, DL2, and MS2 treatments. Additionally, the application of solid digestate in the amount which brought 200 kg N ha^{-1} into the soil (DS2) increased the Pb content in the leaves in relation to the control but decreased the Cr content. The application of manure (MS1 and MS2) and liquid digestate (DL1) reduced the Cr content in the leaves. The growing season was the significant source of variation for the heavy metal content in the enlarged kohlrabi stems and leaves.

Table 7. Heavy metals (mg kg^{-1}) in enlarged kohlrabi stems and leaves (dry matter) as a result of digestates, manure, and mineral fertilizer application.

Treatment	Stem			Leaves		
	Pb	Cr	Cd	Pb	Cr	Cd
Ø	0.193 ^{bc}	1.870 ^{ab}	0.079 ^{a}	0.094 ^{c}	2.351 ^{a}	0.049 ^{a}
DS1	0.114 ^{c}	1.857 ^{ab}	0.032 ^{bc}	0.094 ^{c}	1.945 ^{ab}	0.035 ^{a}
DS2	0.147 ^{bc}	1.521 ^{b}	0.023 ^{c}	0.159 ^{ab}	1.728 ^{b}	0.068 ^{a}
DL1	0.157 ^{bc}	1.835 ^{ab}	0.061 ^{ab}	0.105 ^{bc}	1.796 ^{b}	0.051 ^{a}
DL2	0.304 ^{a}	2.083 ^{a}	0.087 ^{a}	0.106 ^{bc}	1.925 ^{ab}	0.061 ^{a}
MS1	0.288 ^{a}	2.147 ^{a}	0.071 ^{a}	0.157 ^{abc}	1.841 ^{b}	0.042 ^{a}
MS2	0.282 ^{a}	2.051 ^{ab}	0.027 ^{bc}	0.122 ^{bc}	1.615 ^{b}	0.045 ^{a}
NPK	0.220 ^{ab}	1.521 ^{b}	0.057 ^{abc}	0.206 ^{a}	1.969 ^{ab}	0.039 ^{a}
Growing season						
Season I	0.325 ^{a}	1.250 ^{c}	0.050 ^{b}	0.077 ^{b}	1.244 ^{b}	0.058 ^{a}
Season II	0.180 ^{b}	1.807 ^{b}	0.031 ^{b}	0.162 ^{a}	2.139 ^{a}	0.052 ^{ab}
Season III	0.134 ^{b}	2.526 ^{a}	0.083 ^{a}	0.152 ^{a}	2.305 ^{a}	0.036 ^{b}
Source of variation						
T	*	ns	*	*	ns	ns
G	*	*	*	*	*	ns
TxG	*	ns	*	*	*	*

Ø, control; DS1, solid digestate 100 kg N ha^{-1}; DS2, solid digestate 200 kg N ha^{-1}; DL1, liquid digestate 100 kg N ha^{-1}; DL2, liquid digestate200 kg N ha^{-1}; MS1, solid manure 100 kg N ha^{-1}; MS2, solid manure 200 kg N ha^{-1}; NPK, mineral fertilizer. Values followed by different letters are significantly different at $p < 0.05$. Source of variation (ns, not significant; *, significant at significance level $p < 0.05$; T, fertilization treatments; G, growing season; TxG, interaction between treatments and growing season). In the experiment, average dry matter content of enlarged stems was 14.47% and average dry matter content of leaves was 14.77% (based on the dry matter content of 10 separate enlarged kohlrabi stems and leaves).

4. Discussion

Plant-available P_2O_5 and K_2O in the soil in our research did not differ significantly depending on the fertilization treatments (Table 3). The previous findings of Tan et al. [31] showed that digestate application in the amount of 114 and 228 kg N ha^{-1} improved the available P and K levels in the soil. On the other hand, in the research conducted by Panuccio et al. [32] using a pot experiment, the K^+ was not influenced in a sandy loam soil amended with the liquid digestate at the concentrations of 10, 25, and 50% liquid digestate in the total amount of soil. The same authors stated that the amount of K^+ was increased in both soils (loamy sand and sandy loam) amended with fresh solid digestate at the application rates of 50 and 75% of solid digestate to the soil.

Significant season-to-season variability in the concentration of microelements (Fe, Mn, Cu, and Zn) in their available forms was observed. There was a difference in the DTPA-microelement concentration in the soil, depending on whether the treatments were performed with or without organic fertilizers or on the growing season. A lower DTPA-Fe concentration in the soil was observed with the application of organic fertilizers compared to unfertilized control (10 days after fertilizer application) (Table 3), probably due to the formation of insoluble complexes between organic acids (released from organic substrates after being added to the soil) and Fe and Mn [33,34]. On the other hand, earlier reports stated that the application of manure significantly increased the total Fe, Cu, Zn, and Mn contents in soil [35], and the application of digestate (solid or liquid) increased the DTPA extractable Mn fractions [36], which coincides with our results for the increased DTPA-Mn contents in the postharvest soil (Table 3). According to Möller and Müller [37], the conflicting and confusing evidence of microelement availability in the soil treated with digestate shows the importance of taking into consideration all the processes which may affect the availability of microelements, such as the digestate composition, the absorption of elements into the solid fraction, the formation of a complex in solution, and precipitation as carbonate, phosphates, sulfide, and hydroxides. They also concluded that the operating conditions of the digester can affect the availability of microelements from digestates.

In all growing seasons, the application of solid and liquid digestates (both doses), as well as the application of mineral NPK fertilizers, had a positive effect on the total yield of the enlarged kohlrabi stems (Figure 2a). Our results are in agreement with many previous studies, which have also observed a positive effect of digestate application on the yield of different crops, such as corn and wheat [38], red tomato [39], and barley [40]. Digestate contains available N, P, and K, and its soil application may increase the content of chlorophyll in leaves, which stimulates photosynthesis and increases crop yield [41]. In addition, the degradation of organic matter during the anaerobic process could result in producing forms of N, P, and K that are more readily available than those from feedstock [42]. In the research of Ertani et al. [43] concerning the biostimulatory effect of digestates, they found a significant effect of bioactive digestate compounds on maize plants, acting as auxin-like growth promoters. According to Tan et al. [31], long-term digestate application can improve soil fertility and plant yield. The positive effect on increasing kohlrabi yield (enlarged stems and leaves) with manure application was not registered in all growing seasons (Figure 2). Some studies reported that the application of manure did not increase yields [44,45], due to the slow release of N from manure for crop uptake. On the other hand, digestate is characterized by a higher proportion of NH_4-N in the total N content in comparison to that in other organic fertilizers [46]. According to Zavattaro et al. [47], the factors that influence the yield response in the application of manure are the type of manure, the climatic conditions, and the type of crop. The manure used as feedstock for biogas production may contain large amounts of undecomposed straw. Manure and straw application may temporarily induce the biological immobilization of N in soil, which may have a negative effect on N uptake and growth [48]. In all growing seasons, the application of solid and liquid digestates (both doses), as well as the application of mineral NPK fertilizers, had a positive effect on the kohlrabi leaf yield. Digestate contains a large number of active substances, such as monosaccharides, nucleic acids, vitamins, amino acids, and phytohormones (gibberellins), which can promote plant growth [49,50]. In the second growing season (spring 2020, Nemanovci), besides the solid digestate, the liquid digestate also increased the kohlrabi leaf mass, and the mineral fertilizers did as well. Nabel et al. [51] found that fertilizing the *Sida hermaphrodita* plant with digestate resulted in a fivefold increase in biomass yield.

The higher leaf mass obtained in the second season (spring 2020) compared to the other seasons was probably a consequence of the longer days in spring than in autumn. In the research of Caruso et al. [52], the winter–spring production of the *Diplotaxis tenuifolia* plant resulted in a higher leaf surface index, as well as a higher dry matter content compared to the production of autumn–winter crops. Additionally, during the spring growing period, an increase in the soil temperature promotes faster kohlrabi plant growth, due to the more intensive uptake of nutrients from the soil, early maturation, and higher leaf mass. Plants grown during the spring season obtained large leaves and the highest unmarketable yield (although, in the case of kohlrabi plants, young leaves can be used for human consumption in addition to the storage stem). Conversely, during the autumn production of kohlrabi, due to shortening of the day, the limitation of the light, and the lower air and soil temperatures, the plant nutrients from the leaves descend to the upper part of the enlarged storage stem, which increases the yield, so the yield of the edible part is high. In this way, plants are prepared for stressful conditions by depositing nutrients from the leaves into the parts of the plant that provide nutrient storage and survival. Although the only significant difference observed was between planting dates, generally, the aboveground biomass, stem weight, diameter, and yield of the plants planted in autumn were greater than those of the spring planting dates due to the more suitable environmental conditions for kohlrabi [15]. On the other hand, the yield obtained in autumn 2020 was lower than in the first and second seasons, probably due to the lower air temperature (Figure 1) during kohlrabi vegetation than in autumn 2019.

Different studies have investigated the advantages and potential risks of digestate use [32,37,53]. In general, digestates as organic fertilizers are considered a source of plant nutrients and increasers of soil organic matter, but some potential contaminants may be present in them (heavy metals). Usually, organic materials do not contain high levels of heavy metals, but their use can still increase or decrease the content of heavy metals in soil, and this depends on the type of organic fertilizer, the pH value of the fertilizer, the soil type, and many other factors. The application of organic fertilizer in the long term could be a potential source of soil pollution, because of heavy metal accumulation in manure [54]. The concentration of DTPA-Pb, as well as -Cr and -Cd, was influenced by digestates, manure, and mineral fertilizer application (Table 4). The increased concentration of DTPA-Pb (both doses of manure) in the soil (Table 4) could be explained by the addition of the organic fertilizer (Table 2) and by the unfavorable water regime. For instance, Ayaz et al. [55] stated that the application of biochar derived from pig manure digestate increased the heavy metal (Pb and Cr) concentrations in soil under different moisture regimes. In their research, digestate biochar application significantly increased Pb and Cr concentrations in flooded soil moisture conditions, compared to the treatment without biochar application. In our research, the amount of precipitation during the kohlrabi vegetation in 2020 at the Nemanovci site was much higher than the long-term average (Figure 1b). In some treatments, the application of organic fertilizers led to a reduction in the DTPA-Cr and -Cd concentrations in the soil (Table 4). The availability of heavy metals in soil is strongly affected by different factors. One of them is the soil organic matter, which, during the process of the formation of a stable organic fraction, can lead to heavy metal immobilization or adsorption [56]. According to Shan et al. [57], manure decomposition through different microbial processes could increase the humic substances in soil, and according to Yu et al. [58], humic substances have a significant role in forming complexes with metal ions. It has been reported that manure application to a slightly contaminated soil significantly decreased the available Cd and Pb in the soil [59], probably by converting the available metal forms to stable fractions, such as oxide-bound, carbonate-bound, and organic-matter-bound forms of Fe and Mn [60]. Our results showed a different effect of organic fertilizers on the concentration of heavy metals in the soil. For example, the Cd concentration in the soil decreased with the manure (both doses) treatments 10 days after fertilizer application compared to the control, while after the kohlrabi harvest, the Cd decreased with the application of solid digestate in the amount which brought 200 kg N ha^{-1} (DS2) into the soil compared to control. Previous research [61] has shown that with an increase in the soil pH and organic matter, the heavy metal availability generally decreases. It would be expected that the total content of heavy metals in the soil increases or remains stable with the application of organic material. In an 11-year field study, Wierzbowska [62], using different organic materials, found that the total content of heavy metals in the soil was increased by organic amendment compared to control. On the other hand, in the same research, the availability of heavy metals was often not increased. Thus, heavy metal immobilization in the long term seems unlikely [63]. Additionally, Möller and Müller [37] showed a decrease in the heavy metal availability in pot experiments. However, in the research of Tang et al. [64], after 5 years of the application of biogas slurry in wheat and rice production (N rate: 150 kg N ha^{-1} for wheat, 250 kg N ha^{-1} for rice, annually) no increase in the total Pb and Cd contents in the soil was observed. Our study only measured the DTPA available metals in the soil, and not the total element content, so the effect of the organic fertilizers on the total content cannot be commented on.

In terms of the macronutrient content in the enlarged stems (Table 5), a reduced uptake and accumulation of Fe were noticed with organic (DS2, DL2, MS1, and MS2) and mineral fertilizer application. This could be explained by the higher precipitation in spring, which was far above the long-term average (Figure 1b). Wet soil conditions in calcareous soils, for example, are known to cause symptoms of Fe deficiency (chlorosis) in soybeans [65]. In the enlarged kohlrabi stems and leaves, the content of microelements and heavy metals was determined in the following order Fe > Mn > Zn > Cu > Cr > Pb > Cd. In our experiment, this is in line with the content of microelements and heavy metals in digestates and manure, and it is in accordance with other studies [64,66], where the content of heavy metals in wheat and rice was determined in the same order. The mineral composition (Fe, Mn, Cu, and Zn) of the kohlrabi leaves was not affected by the application of digestates and manure compared to the control (Table 6). In a field experiment carried out from 2014 to 2016, Przygocka and Grzebisz [67] observed a considerably higher Zn content in maize grain after digestate application, while in the research of Tang et al. [64], the application of slurry from a biogas plant significantly increased the Zn and Cu contents in rice and wheat. The use of digestate and manure in our research fully satisfied the kohlrabi's needs for the necessary microelements when compared to the recommended limit values of the optimal microelement contents (for Zn, 20–60 ppm; for Mn, 50–150 ppm; and for Cu, 5–12 ppm) in young, fully developed leaves, immediately before the harvest, according to Bergmann [68]. The lead content in the enlarged stems (Table 7) increased with MS1, MS2, and DL2 fertilizer application, and the Pb content in the leaves increased with the NPK treatments, due to the increased soil DTPA-Pb (Table 4) and inputs through organic (Table 2) and mineral fertilizers [69]. This suggests that the heavy metal content in plants can be related to the plant-available fractions in the soil. On the other hand, Tang et al. [64] reported that the heavy metal content in plant roots is related to the total content of heavy metals in the soil rather than to plant-available fractions, and according to Shahid et al. [70], it is probably due to passive transport. A study by Barłóg [66] examined the chemical composition of cattle slurry and digestate, as well as the effect of digestates and manure on trace metal transfer from soil to plants (wheat and barley). These authors concluded that, despite the microelement and Cd content increase due to fertilization with digestate and cattle slurry, the content remained below the European Union's permissible limits, except for the Pb content. In the same research, however, the increased Pb content was influenced by factors other than fertilization. According to the Republic of Serbia's regulations [71], the maximum permitted contents of Pb and Cd for stem vegetables are 0.3 and 0.1 mg kg^{-1} wet weight, respectively. In order to convert to wet weight, the data for the dry matter content are given in the caption under Table 7. In our study, the Pb and Cd contents in the enlarged kohlrabi stems and leaves remained below the maximum permitted limit.

5. Conclusions

The application of digestates (solid and liquid) before planting kohlrabi led to a significant increase in the kohlrabi yield in all growing seasons, as well as the application of mineral NPK fertilizers. Manure could be used to improve the nutritional potential of soil in the next few years, but in the first year, a positive impact on the yield of plants with short vegetation periods, such as kohlrabi, should not always be expected. The use of digestate and manure in the amount of 100 and 200 kg N ha^{-1} in our research fully satisfied kohlrabi's needs for the necessary microelements. Organic fertilizers are complete fertilizers because they contain all the necessary macro- and microelements. The results showed that in kohlrabi production systems, digestates can fully replace mineral NPK fertilizers, achieving stable high yields while maintaining quality. Additionally, in this research, we found that digestates can be used as regular fertilizers, because they either contain nutrients in an easily accessible form and/or are easily mineralized in soil, so nutrients are available in the year of application.

Furthermore, the results clearly indicate that digestates, both solid and liquid fractions (as well as manure), could be utilized as fertilizers in agricultural production with a low risk of contaminating the food chain with heavy metals. This refers to the digestate made from the manure of cattle that are mainly fed with fodder produced on a farm. In any case, the control of soil and the quality of organic fertilizers is necessary and recommended before their application.

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