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ORIGINAL PAPER

EFFECT OF BIOGAS DIGESTATE, INCUBATION, AND NPK FERTILIZATION ON SOME SOIL PROPERTIES AND GROWTH, MINERAL NUTRITION, AND NUTRIENT UPTAKE OF WHEAT*

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Abstract

This study aimed to investigate the effect of biogas digestate (BD) under different incubation times and different NPK levels on the growth and mineral nutrition of wheat. To this aim, 0, 15, and 30 t ha⁻¹ of BD were mixed into the soils. Afterwards, soils were kept at field capacity by irrigating twice a week for 0, 30, and 60 days. Then, the pots were fertilized with full (200, 100, and 125 mg kg⁻¹) and half (100, 50, and 62.5 mg kg⁻¹) NPK doses. The results showed that the available nutrient concentrations increased with the digestate and incubation. Although electrical conductivity (EC) increased with the treatments, it remained below the threshold level for healthy plant growth. Soil pH did not vary with the applications. The combination of full NPK, a digestate dose of 30 t ha⁻¹ and an incubation period of 60 days was the most effective treatment for dry weight (DW). Based on the examined parameters, it can be said that the BD, and a 60-day incubation period in general. The results also showed that NPK fertilization had a stronger effect on plant growth and nutrient uptake applied with digestate.

Keywords: bio-waste, nutrient availability, plant, soil application, soil fertility.

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INTRODUCTION

Biogas digestate (BD), which is a solid-liquid mixture, originates from the fermentation of organic materials. The composition of biogas digestate is generally 93-99% of water and 1-7% of dry matter (LUKEHURST et al. 2010). Although the composition of BD can vary depending on several factors, it contains noteworthy amounts of mineral elements, enzymes, and amino acids, as well as significant amounts of organic matter (OM) with a relatively low C/N ratio. Biogas digestate increases soil carbon content and stabilizes OM. Additionally, it contains a considerable amount of mineral N compared with other organic sources (ALBURQUERQUE et al. 2012). The application of BD also improves water holding capacity, aeration capacity, nutrient retention capacity, cation exchange capacity, and so on in different soils (ROZYLO, BOHACZ 2020). Organic matter in BD also induces microorganism population and increases soil enzymatic activity (JONIEC 2018). Also, it directly increases soil nutrient contents by means of its already dissolved nutrients and minerals released by mineralization. Furthermore, it increases nutrient availability because of chelating compounds formed during decompositions processes (MADER et al. 1997). Recently, BD has been seen as one of the alternative sources and is increasingly used in soil fertility studies. While some studies have stated that BD increases the effectiveness of chemical fertilizers, other studies have reported that BD is as effective as chemical fertilizers on plant growth and some nutrient content (WALSH et al. 2018). FURUKAWA and HASEGAWA (2006) indicated that biogas residue is comparable to NPK fertilizers for spinach and komatsuna production. Also, PANUCCIO et al. (2019) highlighted the potentiality of digestates for the production of cucumber with reduced chemical mineral fertilizer inputs. GLOWACKA et al. (2020) emphasized that BD can be used successfully especially in low fertile soils and also stated that it will reduce the use of chemical fertilizers.

The aims of the present study were to investigate the effectiveness of BD on the plant available soil nutrient concentrations and availabilities under incubated and non-incubated conditions, and to examine the effects of BD on the effectiveness of NPK fertilizers on wheat growth, mineral nutrition, and nutrient uptake.

MATERIALS AND METHODS

Experimental soil

Some properties of the soil are given in Table 1.

Texture	Silty Loam
pH (1:2.5; soil/water)	8.3
EC (1:2.5; soil/water) (dS m^{-1})	0.3
CaCO ₃ (%)	27
OM (%)	1.7
Total C (%)	1.0
Available Nutrients (mg kg ⁻¹)	
Р	5.4
К	298
Ca	6352
Mg	372
Fe	1.5
Zn	0.6
Mn	5.3
Cu	1.5

Some properties of the experimental soil

Biogas digestate

The BD used consisted of 75% cattle manure and 25% alkaloid processing solid waste mixtures. Dry matter (DM) content of BD was 6.97%, OM content of DM was 70.2%. The pH of BD was 7.4 and EC of BD was 8.2 dS m⁻¹. Total N, C, P, K, Ca and Mg concentrations were 18.6, 35, 0.9, 1.2, 3.7 and 1.1 mg g⁻¹ respectively. Total Zn, Fe, Mn, Cu concentrations were 192, 3177, 254 and 896 mg kg⁻¹, respectively.

Experimental setup

The experiment was conducted using 2-kg batches of soil contained in pots under greenhouse condition as 3 replications with a randomized block design. The experiment consisted of 3 incubation periods, IP (0, 30, and 60 days), 3 BD doses (0, 15, and 30 t ha⁻¹; corresponding to 10 and 20 g pot⁻¹), and 2 NPK levels (full NPK and half NPK). The study was performed at two stages as the incubation and the plant growth stage.

Incubation: The soil batches were placed in pots and left for incubation for 0, 30, and 60 days after the application of 0, 15, and 30 tha⁻¹ of BD.

Table 1

Plant growth: After the IP, the pots were fertilized with full and half NPK doses. Full NPK (NPK_p) treatments consisted of 200, 100, and 125 mg kg⁻¹ NPK, respectively. Half NPK (NPK_p) treatments consisted of 100, 50, and 62.5 mg kg⁻¹ NPK. Ammonium nitrate, triple superphosphate, and potassium sulfate were used as fertilizer sources. After NPK fertilization, 15 wheat seeds were sown and later thinned to ten.

Plant, soil, and BD analysis

After 2 months of the growing period (beginning of the heading stage), the plants were harvested by cutting above the soil surface. Total nutrient concentrations in wheat were determined as given in JONES et al. (1991). NaHCO₃ extractable P, NH₄AOC exchangeable K, Ca, and Mg, DTPA extractable Fe, Cu, Zn, and Mn, the OM content of soil and other soil characteristics were measured as given in KACAR (2009). The total C and N contents of BD were determined according to the Dumas method with a LECO-Truspec CN analyzer. Total nutrient concentrations of BD were measured as in plant analysis. The pH and EC values of BD were measured with pH and EC meters. The dry matter content of BD was measured by keeping it at 70°C until reaching stable weight. Organic matter content was calculated through dry-ashing at 550°C for 5 h. Soil and plant analysis results were check using reference soil (GBW07412a:CRMCH, China) and plant (NIST SRM 1515, apple leaves) samples.

The statistical evaluations were made using the MSTAT program. Differences between means were compared using the Duncan's multiple range test.

RESULTS

Soil properties after IP

The effects of the factors on pH (8.2-8.3), Ca (6423-6664 mg kg⁻¹) and Mg (366-418 mg kg⁻¹) were not significant. However, the effects of BD doses and IP on EC, P, K, Fe, Cu, Mn, and Zn showed significant effects (Table 2). The EC values showed significant increments depending on the mean values of IP and BD treatments when compared to the control groups. The soil P concentrations significantly varied with all the factors. The mean values obtained from both individual factors showed that the soil available P concentrations increased by 33% and 51% at 15 and 30 t ha⁻¹ BD doses, respectively. At the same time, it was determined that the P values obtained from the treatments exposed to incubation for 30 and 60 days increased by 32% and 41%, respectively. Based on interaction results, it can be seen that the soil K concentrations significantly varied between 282 mg kg⁻¹ and 356 mg kg⁻¹. The mean values based on BD doses and IP showed that the soil K concentrations under control treatments significantly increased with BD doses and

		BD doses (t ha-1)		
IP (days)	0	15	30	Mean
		EC		
0	0.16	0.16	0.17	0.16B*
30	0.16	0.18	0.20	0.18A
60	0.17	0.19	0.20	0.19A
Mean	0.16B**	0.18AB	0.19A	
		Р	<u> </u>	
0	5.3c***	6.7bc	5.6c	5.9B
30	5.7c	7.5b	10.2a	7.8A
60	6.3bc	8.5ab	10.0a	8.3A
Mean	5.7C	7.6B	8.6A	
		K	·	_
0	282b	310ab	303ab	298B
30	302ab	333ab	356a	330A
60	309ab	346a	342a	332A
Mean	298B	330A	334A	
	·	Fe		
0	1.5	1.8	1.5	1.6AB
30	1.5	1.9	2.0	1.8A
60	1.5	1.4	1.6	1.5B
Mean	1.5B	1.7A	1.7A	
	·	Cu		
0	1.5cd	1.9bc	1.8bcd	1.7B
30	1.6cd	2.4a	2.1ab	2.0A
60	1.4d	1.6cd	1.6cd	1.5B
Mean	1.5B	2.0A	1.8A	
		Mn		
0	5.4	5.9	6.6	6.0AB
30	5.5	7.1	7.1	6.6A
60	5.0	5.4	5.0	$5.1\mathrm{B}$
Mean	5.3B	6.1 <i>AB</i>	6.2A	
		Zn		
0	0.60	0.76	0.72	0.69AE
30	0.64	0.80	0.72	0.72A
60	0.60	0.72	0.60	0.64B
Mean	0.61 <i>B</i>	0.76A	0.68AB	

IP. However, no significant differences were observed between BD doses of 15 and 30 t ha⁻¹ of and incubations of 30 and 60 days. When looking at the means, it was observed that available Fe, Cu, Mn and Zn concentrations increased with BD applications compared to the control, but there was no significant difference between low and high BD doses. Additionally, IP had significant effects on soil Fe, Cu, Mn, and Zn concentrations. Although the available micronutrients showed an increment until day 30 of incubation, afterwards they began to decrease. They even fell below the control values when the incubation was prolonged until day 60. Only the soil Cu concentration significantly varied due to interaction effects (p<0.05).

Plant dry weight (DW) and nutrient concentrations

Plant DW and macronutrient concentrations are given in Table 3. The effects of BD, IP, and NPK fertilization and their interactions significantly affected Table 3

Ар	plications	3	DW	Ν	Р	K	Ca	Mg
		BD_0IP_0	5.1cde*	26ab	1.4dg	42dg	12bcd	2.0be
		$\mathrm{BD_0IP}_{30}$	7.1ad	24abc	1.6cf	43cde	16ab	2.0bcd
		$\mathrm{BD_0IP_{60}}$	6.6be	28a	2.1ab	52b	18a	2.2abc
		$\mathrm{BD}_{15}\mathrm{IP}_{0}$	7.2abc	22ad	1.6cf	37efg	10dg	1.8cf
	NPK_{f}	$\mathrm{BD}_{15}\mathrm{IP}_{30}$	7.9ab	23abc	1.8bcd	43cde	13cd	2.2abc
		$\mathrm{BD_{15}IP_{60}}$	7.8ab	20be	1.5dg	48bc	15abc	2.1ad
		$BD_{30}IP_0$	6.8bcd	24ab	1.4dg	40efg	15abc	1.9be
		$\mathrm{BD}_{30}\mathrm{IP}_{30}$	5.7cde	25ab	2.2a	61a	16ab	2.5a
		$\mathrm{BD}_{30}\mathrm{IP}_{60}$	9.0a	22ad	1.7cde	51b	16ab	2.3ab
NPK×BD×IP		BD_0IP_0	4.6e	20be	1.4dg	48bc	15.0abc	2.1ad
		$\mathrm{BD}_{0}\mathrm{IP}_{30}$	5.4cde	18cde	1.2g	38efg	7.6eh	2.1ad
		$\mathrm{BD_0IP_{60}}$	5.1de	20be	1.3fg	37efg	6.2gh	1.6def
		$\mathrm{BD}_{15}\mathrm{IP}_{0}$	5.1de	17de	1.5dg	38efg	11.0de	2.2abc
	NPK_h	$\mathrm{BD}_{15}\mathrm{IP}_{30}$	5.3cde	16de	1.3efg	37efg	7.8eh	2.1ad
		$\mathrm{BD}_{15}\mathrm{IP}_{60}$	6.3be	15e	1.6cf	36g	6.8fgh	1.4f
		$\mathrm{BD}_{30}\mathrm{IP}_{0}$	6.0be	20be	1.5 dg	36g	10.0def	2.0ad
		$\mathrm{BD}_{30}\mathrm{IP}_{30}$	5.7cde	16de	1.6cf	37efg	5.6 h	1.8cf
		$\mathrm{BD}_{30}\mathrm{IP}_{60}$	6.2be	14e	1.9bc	37efg	5.9 h	1.4f
		BD_0	6.2BC**	26A	1.7A	46B	15A	2.0AB
	NPK_{f}	BD_{15}	7.6A	22BC	1.6AB	43BC	13B	2.0AB
NDKABD		BD_{30}	7.1AB	23AB	1.8A	51A	16A	2.3A
TAT IZVDD		BD_0	5.0D	19CD	1.3C	41C	9.6C	1.9BC
	NPK_h	BD_{15}	$5.6 \mathrm{CD}$	16E	1.5B	37D	8.5CD	1.9BC
		BD_{30}	6.0CD	17DE	1.7A	36D	7.3D	1.8C

Effect of treatments on DW (g pot-1) and N, P, K, Ca, and Mg concentrations (mg g-1)

cont. Table 3

Ар	plications	3	DW	Ν	Р	K	Ca	Mg
		IP_0	6.3 BC***	24 A	1.5 CD	40 BC	13 B	1.9 B
	NPK _f	IP_{30}	6.9 AB	24 A	1.9 A	49 A	15 A	2.2 A
NPK×IP		IP_{60}	7.8A	23 A	1.7 AB	50 A	17 A	2.2 A
		IP_0	5.2 D	$19\mathbf{B}$	1.5 CD	41 B	12 B	2.1 AB
	NPK	IP_{30}	5.5CD	$17\mathbf{B}$	1.4 D	37 C	7.0 C	2.0 AB
		IP_{60}	5.9 CD	$16\mathbf{B}$	1.6 BC	37 C	6.3 C	1.5 C
		BD_0	$4.9d^{\#}$	23ab	1.4d	45b	14a	2.0ab
	IP ₀	BD_{15}	6.1bcd	20bc	1.6bcd	38e	11bc	2.0ab
		BD_{30}	6.4abc	22ab	1.5cd	38e	13ab	2.0ab
	IP_{30}	BD_0	6.2 <i>bc</i>	21abc	1.4d	41cde	12abc	2.0ab
IP×BD		BD_{15}	6.6abc	20bc	1.5cd	40 de	10c	2.1 <i>a</i>
		$\mathrm{BD}_{_{30}}$	5.7cd	21abc	1.9 <i>a</i>	49a	11bc	2.2a
		BD_0	5.8bcd	24a	1.7bc	45b	12abc	1.9ab
	IP ₆₀	BD_{15}	7.1 <i>ab</i>	18c	1.6bcd	42bcd	11bc	1.7b
		BD_{30}	7.6a	18c	1.8ab	44bc	11bc	1.9ab
NDK	NPK_{f}		7.0A****	24A	1.7A	46A	15A	2.1A
NPK	NPK		5.5B	17B	1.5B	38B	8.5B	1.8B
	0		$5.6B^{+}$	23 A	1.5 B	43 A	13 A	0.20
BD	15		6.6A	19 B	1.6 B	40 B	11 B	0.19
	30		6.5A	20 B	1.7 A	44 A	12 AB	0.20
	0		$5.8\mathbf{b}^{\&}$	22 a	1.5 b	40 b	12 a	2.0 a
IP	30		6.2 b	20 b	1.6 ab	43 a	11 b	2.1 a
	60		6.8 a	20 b	1.7 a	44 a	11 b	1.8 b

* NPK×BD×IP,** NPK×BD,*** NPK×IP, # IP×BD, **** NPK, * BD doses, & IP

wheat DW. According to the NPK×BD×IP interaction, the lowest DW was obtained in the soil treated with half NPK but without BD and left to incubation. However, the highest DW was measured from the 60-day incubation with BD of 30 t ha⁻¹ under full NPK application. Looking at the NPK×BD interactions, it was seen that DW values obtained from the NPK+BD applications were higher than those of only the NPK applications for both NPK_f and NPK_h. Dry weights, which were 6.2 g pot⁻¹ in only full NPK (NPK_fBD₀) and 5.0 g pot⁻¹ in only half NPK (NPK_hBD₀) treatments, showed an increment of 23 and 15% and 12 and 20% following the application of NPK+15 and 30 t ha⁻¹. The means of the NPK×IP interaction showed that the lowest DW was obtained from the NPK_hIP₀ treatments. However, the highest DW was obtained under the conditions of 60-day IP of BD with full NPK. When an evaluation was made according to the averages of the IP×BD interactions,

it was seen that the lowest DW value was obtained from the $IP_0 \times BD_0$ application and the highest DW value was obtained from the $IP_{60} \times BD_{30}$ application. The results also showed that BD of 15 and 30 t ha⁻¹ left for 60-day incubations resulted in the highest DW values. The average values clearly showed that there were certain differences between NPK applications, between IP, and between BD doses. The mean DW under full NPK conditions was 27% higher than the means of DW under half NPK conditions. While DW was 5.6 g pot⁻¹ under 0 tha⁻¹ BD conditions, it increased to 6.6 g pot⁻¹ with 15 t ha⁻¹ of BD dose. Plant DW did not vary when the BD dose increased to 30 t ha⁻¹. Although DW increased depending on the IP, the data obtained only from 60 days of incubation showed a significant increase compared to the other doses.

All the individual factors and their interactions significantly affected the plant N concentrations. Based on the general interaction, it was seen that the wheat N concentration was the lowest when 30 t ha⁻¹ of BD was incubated for 60 days followed by half NPK application. On the other hand, the wheat N concentration was the highest with the BD dose of 0 t ha⁻¹ under 60-day incubation with full NPK. According to the NPK×BD interaction, while the highest N was obtained through the combination of full NPK application and 0 t ha^{-1} of BD, the lowest was determined with the combination of half NPK application and 15 tons of BD. Looking at the NPK×IP interactions, it was observed that the plant N concentrations under full NPK were significantly higher than that under half NPK. The wheat N concentrations varied between 18 and 24 mg g^{-1} under the IP×BD interactions. The means of the NPK applications showed that the N concentration of wheat grown under full NPK was 1.37 times higher than that supplied half NPK. The N concentrations of wheat showed a decreasing tendency compared to the control applications with increasing BD doses and prolonged IPs.

The P concentrations of wheat showed significant variations between 1.2 and 2.2 mg g^{-1} depending on general interactions. The average values of the NPK×BD interaction showed that the P concentrations under NPK_bBD₀ applications were the lowest, being the highest under NPK_fBD_{30} treatments. The means of the NPK×IP interactions indicated that the lowest P concentration was recorded under conditions of half NPK+30-day incubation. The highest P was recorded in treatments with full NPK+30-day incubation. According to the means of the IP×BD interactions, the P concentration obtained from $\mathrm{IP}_0\mathrm{BD}_0$ and $\mathrm{IP}_{30}\mathrm{BD}_0$ treatments was the lowest compared to the other treatments. The highest P was determined under the conditions of IP₃₀BD₃₀. Based on the averages of individual factors, full NPK conditions, a BD dose of 30 t ha⁻¹, and 60 days of incubation were the most effective treatments on the wheat P concentration. When looking at the general interactions of individual factors, the concentrations of plant K, Ca, and Mg varied between 36 and 61, 5.6 and 18 and 1.4 and 2.5 mg g^{-1} respectively. The averages of the NPK×BD interactions showed that the application of 30 t ha⁻¹ of BD together with NPK_{ϵ} gave the highest values of the K, Ca, and Mg concentrations. On the other hand, the lowest values were obtained under the application of 30 t ha⁻¹ of BD with NPK_h condition. The average values obtained from the NPK×IP interactions presented that the plants grown under 30 and 60 days of incubation + NPK_f conditions had higher concentrations of K, Ca, and Mg when compared to the others. When evaluated according to the IP×BD interaction, it was seen that the highest K and Mg concentrations in the plant were obtained from the IP₃₀BD₃₀ treatments and the highest Ca concentration was obtained from the IP₀BD₀ treatments. Considering the individual effects of the treatments, it was seen that the conditions with NPK_f were more effective on the K, Ca, and Mg concentrations of the plant than the conditions with NPK_h. No definite tendency was observed in the effects of the other individual factors on the K, Ca, and Mg concentrations of the plant.

The wheat Fe and Cu concentrations determined by the interactions of NPK×BD×IP varied between 62 and 106 mg kg⁻¹ and between 7.4 and 16.3 mg kg⁻¹, respectively (Table 4). According to the NPK×BD interactions,

Table 4

А	pplications		Fe	Cu	Mn	Zn
		BD_0IP_0	88be*	12.4bcd	113abc	38a
		$\mathrm{BD_0IP}_{30}$	87bf	10.6cf	103ae	27be
		$\mathrm{BD}_{0}\mathrm{IP}_{60}$	94abc	13.2bc	115abc	28be
		$\mathrm{BD}_{15}\mathrm{IP}_{0}$	106a	13.2bc	120ab	30abc
	NPK_{f}	$\mathrm{BD_{15}IP_{30}}$	88be	13.4abc	102ae	26bf
		$\mathrm{BD}_{15}\mathrm{IP}_{60}$	93ad	14.6ab	125a	28ad
		$BD_{30}IP_0$	96ab	15.3ab	109ad	33ab
		$\mathrm{BD}_{30}\mathrm{IP}_{30}$	75ej	15.2ab	110abc	28ad
		$\mathrm{BD}_{30}\mathrm{IP}_{60}$	80ch	16.3a	116ab	27be
NPK×BD×IP		$\mathrm{BD}_{0}\mathrm{IP}_{0}$	71gj	10.1dg	118ab	21cf
		$\mathrm{BD_0IP}_{30}$	71gj	11.3cde	115abc	21cf
		$\mathrm{BD_0IP_{60}}$	64ıj	11.3cde	105ad	22cf
		$\mathrm{BD}_{15}\mathrm{IP}_{0}$	79dı	10.0dg	96ae	20def
	NPK_h	$\mathrm{BD_{15}IP_{30}}$	72fj	8.4efg	93ae	18ef
		$\mathrm{BD_{15}IP_{60}}$	62j	7.4g	76de	16f
		$\mathrm{BD}_{30}\mathrm{IP}_{0}$	81cg	8.1fg	92be	19def
		$\mathrm{BD}_{30}\mathrm{IP}_{30}$	62j	8.7efg	82cde	19def
		$\mathrm{BD}_{30}\mathrm{IP}_{60}$	66hıj	$7.8 \mathrm{fg}$	71e	19def
		BD_0	97A**	13.6AB	114AB	34A
NPK×BD	NPK_{f}	BD_{15}	83BC	13.0B	105ABC	27B
	1	BD ₃₀	89B	14.7A	119A	28B

Effect of treatments on Fe, Cu, Zn, and Mn concentrations (mg kg-1)

1	0	6	

cont.	Table	4
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	Applications		Fe	Cu	Mn	Zn
		BD_0	77C	9.4C	102BC	20C
NPK×BD	NPK _h	BD_{15}	68D	9.5C	97CD	19C
		BD ₃₀	64D	8.8C	84D	19C
		IP_0	90 AB ***	12.0 B	110 A	31 A
	NPK _f	IP ₃₀	96 A	13.7 B	116 A	28 A
NPK×IP		IP_{60}	84 B	15.6 A	112 A	30 A
		IP_0	69 C	10.9 B	113 A	22 B
	NPK	IP ₃₀	71 C	8.6 D	88 B	18 B
		IP_{60}	70 C	8.2 D	82 B	19 B
		BD_0	80 <i>bc</i> #	11.3	116a#	30a#
	IP ₀	BD_{15}	79 <i>c</i>	11.0	109 <i>ab</i>	24ab
		BD ₃₀	79 <i>c</i>	12.2	110 <i>ab</i>	25ab
		BD_0	92 <i>a</i>	11.6	108 <i>ab</i>	25ab
IP×BD	IP ₃₀	BD_{15}	80 <i>bc</i>	10.9	98ab	22b
		BD_{30}	78cd	11.0	100 <i>ab</i>	22b
		BD_0	89ab	11.7	100 <i>ab</i>	26ab
	IP_{60}	BD_{15}	69d	12.0	96ab	23ab
		BD_{30}	73cd	12.1	94 <i>b</i>	23b
NDZ	NPK _f		90A****	13.8A	113A	30A
INI K	NPK _h		70B	9.3B	94B	19B
	0		87 A +	11.5	108	27 A
BD	15		76 B	11.3	101	23 B
	30		77 B	11.8	101	24 B
	0		79 b &	11.5	112 a	26 a
IP	30		83 a	11.2	102 b	23 b
	60		77 b	11.9	97 b	24 ab

* NPK×BD×IP, ** NPK×BD, *** NPK×IP, # IP×BD, **** NPK, * BD doses, & IP

the lowest micro-element concentrations were measured under the conditions of NPK_hBD₃₀ generally. While the highest Fe and Zn concentrations were measured from the NPK_fBD₀ interaction, the highest Cu and Mn concentrations were determined from the NPK_fBD₃₀ combinations. The NPK×IP interactions indicated that the highest Fe and Cu values were reached under the conditions of NPK_fIP₃₀ and NPK_fIP₆₀, respectively. While the IP×BD interaction had no effect on the Cu concentration of the plant, the highest Fe value was obtained from the IP₃₀BD₀ treatments. Full NPK doses were the most effective treatments for both nutrients. According to the BD doses, the highest Fe was found under the control dose. The effects of BD on the Cu concenter

trations were not significant. While the most effective incubation time was 30 days for the Fe concentrations, the Cu concentrations were not affected by the IP. The interactions of all of the individual factors showed different effects on the wheat Mn and Zn concentrations (Table 4). The plant Mn and Zn concentrations under these interactions varied from 71 to 125 mg kg⁻¹ for Mn and from 16 to 38 mg kg⁻¹ for Zn. Based on the NPK×BD interactions, the Mn and Zn concentrations of wheat under NPK_b and all BD doses were significantly lower under NPK_c conditions. According to the NPK×IP interactions, the highest Fe and Cu concentrations were obtained from the combination of NPK, IP30 and NPK, IP60 condition. Plant Mn and Zn concentrations under all incubations and with full NPK doses were effected similarly and these nutrient concentrations were significantly higher than in the treatments with half NPK doses, generally. While the IP×BD interaction had no effect on the Cu concentration of the plant, the highest Fe value was obtained from the $IP_{30}BD_0$ treatments. The plant Mn and Zn concentrations measured from the IP_0BD_0 treatments were found to be the highest when compared to the other treatments included in the IP×BD interactions. The means of individual factors indicate that NPK_f had a stronger effect on the wheat Mn and Zn concentrations when compared to NPK_b. While BD doses had no effect on the Mn concentration, 0 dose of BD was the most effective application on the plant Zn concentration. In terms of the IP, it was seen that the wheat Mn and Zn concentrations in non-incubated treatments were the highest.

Nutrient uptakes

Almost all the factors had a significant effect on the N uptake of wheat (Table 5). Looking at the general interaction including all the factors, the amount of N uptake was the highest in the combination of 60-day IP with 30 t ha⁻¹ of BD and full NPK. On the other hand, the lowest N uptake was recorded from the combination of 30 days IP, 15 t ha⁻¹ of BD and half

Table 5

Applications		N	Р	K	Ca	Mg	
		BD_0IP_0	133cf*	7.1de	214e	62dg	10.2cg
		BD_0IP_{30}	170abc	11.4abc	305bcd	114b	14.2bcd
		$\mathrm{BD}_{0}\mathrm{IP}_{60}$	185ab	13.9ab	343bc	119ab	14.5bc
		$BD_{15}IP_0$	158ad	11.5abc	266cde	71cde	13.0bf
NPK×BD×IP	NPK_{f}	$\mathrm{BD_{15}IP_{30}}$	182ab	14.2ab	340bc	103bc	17.4ab
		$\mathrm{BD_{15}IP_{60}}$	156ad	11.7abc	374ab	117ab	16.4ab
		$BD_{30}IP_0$	163ad	9.5cde	272cde	102bc	12.9be
		$\mathrm{BD}_{30}\mathrm{IP}_{30}$	142be	12.5abc	348bc	91bcd	14.3bc
		$BD_{30}IP_{60}$	198a	15.3a	459a	144a	20.7a

Effect of treatments on N, P, K, Ca, and Mg uptakes (mg pot-1)

cont.	Table	5	

A	oplications	3	N	Р	K	Са	Mg
		BD_0IP_0	92fg	6.4e	221de	69cf	9.7dg
		BD_0IP_{30}	97fg	6.5e	205e	41efg	11.3cg
NPK×BD×IP		BD_0IP_{60}	102efg	6.6de	189e	32g	8.2g
		$BD_{15}IP_0$	87g	7.7de	194e	56efg	11.2cg
	NPK_h	$\mathrm{BD}_{15}\mathrm{IP}_{30}$	85g	6.9de	196e	41efg	11.1cg
		$\mathrm{BD_{15}IP_{60}}$	95fg	10.0bcd	227de	43efg	8.8fg
		$BD_{30}IP_0$	120dg	9.0cde	216e	60dg	12.0cg
		$\mathrm{BD}_{30}\mathrm{IP}_{30}$	91fg	9.12cde	211de	32g	10.3cg
		$\mathrm{BD}_{30}\mathrm{IP}_{60}$	87g	11.8abc	229de	37fg	8.7efg
		BD_0	161A**	10.5AB	285B	93B	12.4B
	NPK_{f}	BD_{15}	167A	12.2A	327A	99B	15.2A
NDK×PD		BD_{30}	163A	12.8A	362A	114A	16.3A
NPK×DD		BD_0	95B	$6.5\mathrm{D}$	205C	48C	9.5C
	NPK_h	BD_{15}	90B	8.4C	207C	48C	10.6C
		BD_{30}	102B	10.2B	216C	44C	10.8C
NPK×IP	NPK _f	IP_0	151 B***	9.5 B	252 C	82 C	12.0 C
		IP_{30}	166 AB	13.1 A	338 B	104 B	$15.2\mathbf{B}$
		IP_{60}	179 A	13.3 A	390 A	$133\mathbf{A}$	17.2 A
	NPK_{h}	IP_0	99 C	7.8 BC	213 CD	62 D	10.9 C
		IP_{30}	94 C	7.7C	204 D	39 E	11.0 C
		$\mathrm{IP}_{_{60}}$	94 C	9.4 B	218 CD	37 E	8.9 D
		BD_0	$113b^{\#}$	6.9c	221d	69b	9.8 d
	IP_0	BD_{15}	122ab	9.8b	232cd	67b	12.2 bcd
		BD_{30}	141 <i>a</i>	9.6b	243cd	83ab	12.8 abc
		BD_0	130ab	8.7bc	254bcd	74ab	12.4 ad
IP×BD	IP_{30}	BD_{15}	132ab	9.9b	264 bcd	66b	13.9 ab
		BD_{30}	120ab	10.8ab	279bc	63b	12.5 ad
		BD_0	139a	9.9b	261bcd	70ab	11.0 <i>cd</i>
	IP_{60}	BD_{15}	128ab	11.4ab	298a b	78ab	12.1 bcd
		BD_{30}	137a	13.7a	334a	84a	14.4 a
NDZ	NPK_{f}		168A****	11.9A	322A	105A	14.7A
NEK	NPK_{h}		94 <i>B</i>	8.3B	209B	47B	9.9B
	0		129	8.4 B +	241 B	73	11.2 B
BD	15		125	10.6 A	264 AB	73	12.5 A
	30		130	11.1 A	286 A	78	13.0 A
	0		128 ab &	8.7 c	232 c	70 b	11.6 b
IP	30		124 b	9.9 b	267 b	68 b	13.0 a
	60		136 a	11.6 a	299 a	75 a	12.2 a

* NPK×BD×IP, ** NPK×BD, *** NPK×IP, # IP×BD, **** NPK, * BD doses, & IP

NPK. When an evaluation was made according to the means of the NPK×BD interaction, it was seen that the plants in NPK_{f} and the plants in NPK_{h} were collected in two separate groups, and the plants in NPK, variants took up much more N. The wheat N uptake under the NPK×IP interactions was the highest when full NPK was applied after 60-day IP. According to the evaluation based on the IP×BD interactions, there were no significant differences among most of the treatments. However, the uptake of N in $IP_0 \times BD_0$ treatment remained the lowest when compared to the others. The means of individual factors showed that N removal under NPK_{f} conditions was 1.79-fold higher than under NPK_b conditions. The uptake of N by wheat increased at 60-day incubation conditions; however, BD doses did not show a significant effect on N uptake. The wheat P uptake showed a wide variation between 6.4 mg pot⁻¹ and 15.3 mg pot⁻¹. When looking at the means of each interaction, it could be seen that the lowest mean P uptake was recorded from the $NPK_{b}BD_{0}$, $NPK_{b}IP_{30}$, and $IP_{0}BD_{0}$ treatments, whereas the highest P uptake appeared in the treatments of $NPK_{f}BD_{30}$, $NPK_{f}IP_{60}$, and $IP_{60}BD_{30}$. The averages of individual factors demonstrated that plants grown under $\mathrm{NPK}_{\!_{\rm f}}$ took 43% more P than under $\mathrm{NPK}_{\!_{\rm h}}.$ In addition, the wheat P uptakes increased with the increase of BD doses and IP. According to the general interaction, it could be seen that the lowest and the highest K, Ca, and Mg uptakes were determined from the same combinations. The lowest K, Ca, and Mg uptake, at the values of 189, 32, and 8.2 mg pot^{-1} respectively, was obtained from the NPK_bBD₀IP₆₀ combination, while the highest uptake values were obtained from the NPK_fBD₃₀IP₆₀ combination, at 459, 144, and 20.7 mg pot⁻¹, respectively. Depending on the interactions of two factors (NPK×BD, NPK×IP, and IP×BD), the highest P, K, Ca, and Mg uptakes were reached at the combinations of $NPK_{f}BD_{_{30}},\,NPK_{f}IP_{_{60}}$, and $IP_{_{60}}BD_{_{30}}.$ In terms of N uptakes, $NPK_{f}BD_{15}$, $NPK_{f}IP_{60}$ and $IP_{0}BD_{30}$ were the most effective combinations. Based on a general assessment, it was determined that the most effective application was NPK, and the amount of N, P, K, Ca, and Mg taken by the plant under these conditions was 78, 43, 54, 124, and 48% more than under NPK, conditions, respectively. According to the average effects of BD doses, the P, K and Mg uptakes of wheat under both BD doses were significantly higher than in the control treatment. However, the plant N and Ca uptakes were not affected by BD doses. As for IP, while the most effective IP on the plant macronutrient uptakes was 60 days of IP, generally, 0 day incubation was the least effective treatment in general.

The micronutrient uptakes of wheat are shown in Table 6. The plant Fe and Cu uptakes related to the general interaction were the highest under the NPK_fBD₁₅IP₀ and NPK_fBD₃₀IP₆₀ conditions, respectively. As for the NPK×BD interactions, the plants took up more Fe and Cu under NPK_f conditions for all BD doses when compared to NPK_h treatments. Significantly higher Fe and Cu uptakes were measured with NPK_f in the NPK×IP combinations. Considering the data obtained from the IP×BD interactions, it was observed that the most effective combination for the Fe uptake was IP₃₀BD₀ and the

Table 6

	Effect of t	reatments on	re, ou, mii, a	inu zii uptakes	s (µg por)	
Applications			Fe	Cu	Mn	Zn
		BD_0IP_0	449cf *	63fi	576cg	193bc
		BD ₀ IP ₃₀	618abc	75dh	731be	191bc
		BD_0IP_{60}	620abc	87cf	759bcd	182bc
		BD ₁₅ IP ₀	763a	95cde	864ab	219ab
	NPK_{f}	$BD_{15}IP_{30}$	695ab	106bc	806abc	206abc
		$BD_{15}IP_{60}$	725be	114cg	975bf	222cd
		$BD_{30}IP_0$	653abc	104bcd	741bf	226ab
		$\mathrm{BD}_{30}\mathrm{IP}_{30}$	428ad	87b	627ab	160ab
		$\mathrm{BD}_{30}\mathrm{IP}_{60}$	720ab	147a	1044a	246a
NPK×BD×IP		BD_0IP_0	327f	471	543dg	98e
		BD ₀ IP ₃₀	383ef	61ghı	621cg	114e
		BD ₀ IP ₆₀	326ef	58e1	536bg	113de
		BD ₁₅ IP ₀	403def	51hı	490efg	101e
	NPK _h	$BD_{15}IP_{30}$	382ef	451	493efg	94e
		$BD_{15}IP_{60}$	391ef	471	479g	103e
		BD ₃₀ IP ₀	486def	491	552fg	112e
		$BD_{30}IP_{30}$	353def	50hı	467dg	107de
		$BD_{30}IP_{60}$	409def	481	440g	118de
	NPK,	BD ₀	601A**	84B	707B	210A
		BD_{15}	631A	99A	798AB	205A
NDZ		BD ₃₀	632A	104A	845A	197A
NPK×BD		BD_0	385B	47C	510C	100B
	NPK _h	BD_{15}	381B	53C	543C	108B
		BD ₃₀	384B	53C	504C	115B
		IP ₀	567 A***	76 C	693 BC	193 B
	NPK_{f}	IP ₃₀	662 A	95 B	800AB	$195\mathbf{B}$
NPK×IP		IP_{60}	655 A	122 A	874 A	231 A
		IP ₀	359 B	57 D	588 CD	112 C
	NPK _h	IP ₃₀	391 B	47 D	484 D	99 C
		IP_{60}	413 B	48 D	484 D	111 C
		BD ₀	392c [#]	55d	568b	145bc
	IP_0	BD ₁₅	482abc	67cd	665ab	146bc
		BD ₃₀	506ab	71 <i>b</i>	704 <i>ab</i>	159ab
IL×RD		BD_0	570a	72bc	670ab	156ab
	IP ₃₀	BD_{15}	528ab	72bc	647 <i>ab</i>	145bc
	50	BD ₃₀	445bc	63cd	570b	128c

Effect of treatments on Fe, Cu, Mn, and Zn uptakes ($\mu g \ \text{pot}^{\text{-1}}$)

cont. Table 6

Applications			Fe	Cu	Mn	Zn
IP×BD	IP_{60}	BD_0	516ab	68 <i>c</i>	580ab	151abc
		BD_{15}	500abc	85ab	682ab	166ab
		BD_{30}	555a	92a	714a	176 a
NPK	NPK_{f}		630A****	97A	791A	207A
	NPK _h		385B	51B	517B	107 <i>B</i>
BD	0		487	64 B +	605	151
	15		502	75 A	667	152
	30		501	77 A	657	153
IP	0		458 b &	67 b	650	151 b
	30		515 a	69 b	632	144 b
	60		524 a	81 a	660	165 a

* NPK×BD×IP, ** NPK×BD, *** NPK×IP, # IP×BD, **** NPK, * BD doses, & IP

most effective application for the Cu uptake was $IP_{60}BD_{30}$. In terms of individual factors, NPK_f conditions and 60 days of IP were the most effective treatments for the Fe and Cu uptakes. While BD doses had no significant effect on the Fe uptake, 15 and 30 t ha⁻¹ doses of BD were more effective than the control on the Cu uptake. General interactions of 3 factors indicated that the most effective combination on both the Mn and Zn uptakes was NPK_fBD₃₀IP₆₀. According to the means of the NPK×BD interactions, all BD doses under NPK_f fertilization resulted in higher Fe and Zn uptakes. As for the NPK×BD and IP×BD interactions, the plants took up more Mn and Zn under the combinations of NPK_fIP₆₀, and IP₆₀BD₃₀. When considering the averages of individual factors, NPK_f for both nutrient uptakes was more effective than NPK_h. The doses of BD and the IP did not affect the plant Mn uptakes. While BD doses did not affect the Zn uptake, the 60-day IP was the most effective on the Zn uptake.

DISCUSSION

It was observed that the pH values of all soils exposed to BD applications and different IPs did not change during incubation and the measured values were close to the control group. Similarly, GóMEZ-BRANDÓN et al. (2016) found nonsignificant differences among the treatments before and after the application of digestate. ODLARE et al. (2008) did not find a significant change in the pH after 4-year-long biogas residue application, either. As it is known, basic soil properties such as soil pH are not easily changeable properties. Changing the pH of soils is not possible except in some special cases, such as liming, sulfur application, or long-term, one-way chemical fer-

tilizer applications. The alkaline characteristic of BD used for the experiment might be the reason for its ineffectiveness on soil pH. It was stated that because of its alkalinity, BD causes an increase in pH especially in acid soils, while it is not very effective in alkaline soils (LOSAK et al. 2014). Electrical conductivity was influenced by doses of BD and IP. Nonetheless, in any of the treatments, the EC values were far below the threshold level for healthy plant growth (HERRERO, PÉREZ-COVETA 2005). Another study demonstrated that, depending on BD application and IP, the EC value of the soil could be 1.5 to 2 times higher than in the control (GÓMEZ-BRANDÓN et al. 2016). The reason why there was not such a difference in our study could be the low EC value of the BD used. When compared to the control, the available nutrients such as P, K, Fe, Cu, Mn, and Zn showed an increase with BD doses. Similar results have been reported by MAKADI et al. (2012), and GLOWACKA et al. (2020). This can be explained by the fact that a significant part of the total nutrients in BD is transferred to the soil in plant-available forms. As indicated by Koszel and LORENCOWICZ (2015), digestate resembles mineral fertilizers since N, P, and K nutrients are easily available to plants. CHIEW et al. (2015) said that the use of digestate increases the content of macro- and microelements in soil and plants. Plant-available nutrient concentrations increased with up to 30 days of incubation in general. This may be attributed to the mineralization of nutrients, as it is explained in different studies conducted on different organic materials (ERDAL, EKINCI 2020). Furthermore, the transformation of total nutrients to plant-available forms during the incubation periods may have contributed to the increase of available nutrients (KIRCHMANN, WITTER 1992). The release of chelating compounds, such as protein, humic and fulvic-like substances, during the incubation might be another reason for the increase of available nutrient concentrations in the soil (Young et al. 2018). It was observed that the available microelement concentration in the soils considerably decreased during 60-day incubation. One possible reason for this can be the occurrence of insoluble organo-mineral complexes in the soil (ERDAL, EKINCI 2020) due to prolonged incubation. Also, the higher P concentration under 60 days of incubation may have depressed the availability of micronutrients in the soil solution by building slightly soluble complexes (VAGO et al. 2009). The increase in EC with incubation may be explained by the release of salt-forming cations during incubation. In terms of the DW of plants, it was observed that the DW obtained after the application of NPK, was higher than following the fertilization with NPK_h. Plant DWs increased due to the increase in BD doses and IP compared to the control treatments. These results are in agreement with previous studies (SOGN et al. 2018, GŁOWACKA et al. 2020). It is thought that an increase in plant DWs depending on the application of digestate is closely related to the BD doses and incubations. As a matter of fact, the increase in the amount of available nutrients, such as P, in the soil at the end of these two treatments is one of the factors that increase plant growth. For instance, insufficient P levels under the control conditions increased up

to sufficient levels due to BD application and incubation. As it has been stated, digestates promote plant growth because they contain bioactive substances such as humic-fulvic acids, free amino acids, phytohormones, etc. (Yu et al. 2010). It is an expected result that DW after the application of NPK, is higher than after NPK_b, and this can be associated with the higher and immediate availability of chemical fertilizer nutrients. Looking at the NPK×BD interactions, it was seen that the DWs obtained from the combined applications of NPK and BD were higher than that of only NPK applications. As in DW, the amount of nutrients taken from the soil was higher when NPK and BD were applied together. Looking at the DM increases, we can conclude that the BD applications increased the efficiency of NPK by 19% and 16% on average under full and half NPK doses, respectively. In various studies, it is stated that liquid digestate+mineral fertilizer has stronger influence on biomass production and plant nutrition (EHMANN et al. 2018). As stated above, BD owing to its own properties affecting soil fertility, influences plant growth directly or indirectly (WALSH et al. 2018). At the same time, highly available nutrient concentrations dissolved in the liquid part or the mineral elements released as a result of the mineralization of organic matter in the solid part can increase plant growth by combining with NPK from chemical fertilizers. As reported in several studies, high concentrations of organic matter and mineral nutrient concentrations increase plant growth (LOSAK et al. 2016, DAHLIN et al. 2017).

When an evaluation of the results is made, it emerges that some nutrient concentrations increased depending on BD applications and IP. These results can be attributed to the use of available nutrients in the applied BD by the plant, and by increasing the usefulness of nutrients in the soil due to BD applications and incubation (VANEECKHAUTE et al. 2013). In some cases, nutrient concentrations do not show parallel relationships with plant growth, and even higher nutrient concentrations are measured when plant growth is lower. These results can be explained by higher concentrations of nutrients due to retarded plant growth (JARRELL, BEVERLY 1981). The concentration or dilution of nutrients depending on the growth of a plant can be interpreted by looking at the amounts of nutrients taken up by the plant. As it can be seen from the results, the plant nutrient uptake had a similar tendency to the plant DW. Nutrient uptake generally increased with the increase of DW (ERDAL, EKINCI 2020).

CONCLUSION

Biogas digestate and the incubation period did not affect the soil pH, while a small increase was observed in electrical conductivity. Approximately all the nutrients in the soil showed a significant increase under the influence of biogas digestate and incubation. As for plant growth, the most effective combination was 60-day incubation of 30 tha⁻¹ of biogas digestate together with full NPK dose application (NPK_tBD₃₀IP₆₀). Looking at the examined parameters, full NPK, 15 t ha⁻¹ dose, and 60 days of incubation were the most favorable individual treatments. The research also proved that the efficiency of NPK on plant growth and mineral nutrition was higher when used together with biogas digestate.

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