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Effect of Biogas Digestate on Growth and some Potentially Toxic Elements Conentrations of Wheat

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THIS STUDY aimed to investigate the effect of biogas digested (BD) on the growth and some potential toxic element (B, Cd, Cr, Ni, Co, and Pb) concentrations of wheat grown under greenhouse conditions. For this reason, 0, 15, and 30 tha^{-1} of BD were applied to the soils and incubated for 0, 30, and 60 days. After the end of the incubation period wheat seeds were sawn and left for growth for 2 months. The results indicated that BD dosages and incubation periods increased plant dry weight. Applications of incubated BD did not affect B, Cd and Co concentrations of wheat, while Cr and Ni concentrations decreased generally with the BD dosages. Similarly, incubation showed a decreasing effect on Pb concentration of wheat. Plant Co, Cr, and Pb uptakes did not vary with the applications of BD, other element uptakes increased in the parallels of plant dry weights. The element concentrations of wheat were in the ranges of acceptable levels. So, it was concluded that the application of BD did not pose a risk for potentially toxic elements on the growth of the wheat.

Keywords: Agricultural wastes, Biosolids, Plant growth, Toxicity.

Introduction

Due to the increasing world population, the need and consumption of energy are constantly increasing. Depending on this demand, efforts for finding alternative energy sources is increased as well. Biogas is one of the famous and environmental friendly energy sources. Biogas is produced with the anaerobic fermentation of various organic materials based on plants and animals. After biogas production, a waste named BD containing 93–99% of water and 1-7% of dry matter (Lukehurst et al., 2010; Namli et al., 2020) is obtained. Digestate canalso be defined as liquid from anaerobic decomposition of organic wastes. It contains considerable amounts mineral nutrients such as N, P, K and others. Because of rapid absorbtions of mineral nutrients in it, BD resembles easily available nutrient containing chemical fertilizers. Du to it'sorganic matter content BD plays a positive role on physicochemical properties of applied soils (Koszel & Lorencowicz, 2015). Chiew et al. (2015) say that the use of BD increases the macro and microelements contents in the soil and plants. Obtained BD is a valuable organic fertilizer having noteworthy amounts of mineral elements, enzymes, and amino acids, as well as significant amounts of organic matter with a relatively lower C/N ratio (Kılıç, 2011). The application of BD improves soil water holding capacity, aeration capacity, nutrient retention capacity, cation exchange capacity, and so on in different soils (Alaboz et al., 2021). By changing the pH of the soil, BD can also increase the availability of nutrients in some soils (Mader et al., 1997). In many studies, the effectiveness of BD on plant mineral nutrition, growth, and yield has been demonstrated (Barbosa et al., 2014; Walsh et al., 2018, Yaraşır et al., 2018). Kouřimská et al. (2012) indicated that BD improves soil fertility, plants quality and their immunity to biotic and abiotic agents the quality and yield of vegetables. To sustain agricultural production, it became an important issue to examine the effects of different organic wastes on soil fertility parameters (El-Ramady et al., 2020). At the same time, proper disposal of these wastes has become very important in terms of protecting natural resources

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and environmental pollution (Gökcan, 2012). Although there are many direct or indirect positive effects of BD on soil fertility and plant nutrition, it became a research subject whether BD had any potential to create any toxicity on plants or not. The risk of pollutants in the biosolids or organic wastes is one of the factors limiting their use in soils (Głowacka et al., 2020).

The present study aimed to investigate the effects of different dosages of BD on wheat growth and some potentially toxic element (B, Cd, Cr, Ni, Co, and Pb) concentrations. Also it was aimed to examine these effects under incubated and non incubated conditions.

Materials and Methods

Soil properties

The soil used for the experiment is a silt loam texture and has a slightly alkaline character (pH: 8.0;1/2.5 soil/water) without salinity problem (EC: 0.2 dSm^{-1};1/2.5soil/water). It has high $CaCO_3$(270 mg g^{-1}) and low organic matter (17 mg g^{-1}) .

The values of pH and EC were measured (1:2.5 soil/water mixture, w/w) using pH and EC meters (Hanna HI 2550) (Peech, 1965). Texture and $CaCO_3$ were determined as described by Bouyoucos (1951) and Allison & Moodie (1965), respectively. Organic matter content was measured according to Walkley & Black (1934) method. Plant available P was measured according to the molybdophosphoric blue color method by means of spectrophotometer (Shimadzu UV 1208) (Olsen, 1954). Exchangeable K, Ca, and Mg were determined using AAS (Varian 240 FS) after NH_4AOC extraction (Jackson, 1967). DTPA extractable Fe, Cu, Zn, and Mn, concentrations were measured using AAS as described in Lindsay & Norvell (1969). Soil B concentrations were determined calorimetrically after the extraction in 0.1 m $CaCl_2$ (Sillinpaa, 1982). Total amount of heavy metals (Ni, Cr, Co, Cd and Pb) in the soil were measured using ICP-AES (Thermo, ICAP 6300 DUO)after the wet digestion methods (Khan & Frankland, 1983). Some other characteristics containing nutrient and element concentrations of the soil is given in result section.

Biogas digestate (BD)

The digestate used for the experiment was obtained from the biogas and compost laboratory, Agricultural Machinery and Technologies Department, the Faculty of Agriculture, Isparta, Turkey (37°57′24″N 30°57′39″W). The BD used consisted of 75% cattle manure and 25% alkaloid processing solid waste mixtures. Dry matter (DM) content of BD was 69.7 mg g^{-1}, OM content of DM was 702 mg g^{-1}. The pH of BD was 7.4 and EC of BD was 8.2 dSm^{-1}.

The total C and N contents of BD were determined according to the Dumas method with a LECO-Truspec CN analyzer (Kirsten, 1983). The pH and EC values of BD were measured directly using pH and EC meters. The dry matter content of BD was measured by keeping it at 70°C until it reached a stable weight. Organic matter content was calculated through dry-ashing at 550°C for 5 h. Total element concentrations of BD were measured with the same procedures used for the plant analysis. Some other characteristics of the BD is given in the result section.

Experimental design

The experiment was conducted in 2019 at the Department of Soil Science and Plant Nutrition, the Faculty of Agriculture, Isparta University of Applied Sciences, Isparta, Turkey.The experiment was conducted using 2kg soil contained in pots under greenhouse conditions. The experiment consisted of three incubation periods (IP) (0, 30, and 60 days), three BD dosages (0, 15, and 30 t ha^{-1}), and 3 replicates . The study was performed at two stages; incubationstage thenplant growth stage.

At the incubation stage 0, 15, and 30 t ha^{-1} of BD were mixed into soils then were left for incubation for 0, 30, and 60 days. During the IP, the pots were irrigated at 60% of the water holding capacity. In this stage it was aimed to let the organic matter in the BD mineralized.Also it was assumed that the nutrients in the soil will be more available with the products released from BD during incubation time. There is not any results related to this stage.

At plant growth stage: After whole incubation periods were ended the pots were fertilized with 200 mg kg^{-1} N, 100 mg kg^{-1} P, and 125 mg kg^{-} K as basal fertilization using ammonium nitrate, triple superphosphate, and potassium sulfate fertilizers. After that the soils were mixed with hand and 15 wheat seeds (*Triticum aestivum L.*, bread wheat, cvs.; Tosunbey) were sown. The plants were thinned to tenafter germination. The plant growth period lasted for two months. After the growing period, the plants were harvested over the soil surface. The plants were washed with tap and distilled water, dried at 65–70°C for 48 h, weighed, and grounded. Then, 0.5 g of plant samples was put in porcelain cups and dryashed at 550°C for 5 h. The ash was dissolved in 3 ml concentrated HCl and filtrated, and the final volume was brought up to 50 ml with deionized water (Kacar & Inal, 2008). All elements in the supernatant were measured using ICP-AES at recommended detection limits (0.0400, 0.0072, 0.0010, 0.0022, 0.0035 mg l^{-1} for B, Pb, Cd, Cr and Ni, respectively)

MSTAT program was used for statistical evaluations of the results and Duncan's multiple range test was used to compare means.

Results

Description of the soil and BD used for the experiment

Some initial available and extractable nutrients and total heavy metal concentrations of the soil are given below (Table 1). As indicated there soil is poor in terms of N, P, Zn and Mn concentrations.It has sufficient amounths for K, Ca, Mg, Fe and Cu (Alpaslan et al., 1998). As for total heavy metal concentrations, it contains lower heavy metals than permissible levels that indicated in Osmani et al. (2015) and Tumanyan et al. (2019). The soil heavy metal concentrations are also below the threshold levels indicated by soil soil pollution control regulation in Turkey (Anonymous, 2005).

Total element concentrations of BD is given in Table 2. Looking at the amount of potentially toxic elements in digestate used in this research are quite below the threshold levels given by different countries and organizations for organic wastes and biosolids used for agricultural purposes (Teglia et al., 2011; Collivignarelli, 2019). Different countries and organizations developed their own rules indicating permissible levels of some heavy metals in organic residues and biosolids used for agricultural purposes (Tables 3, 4). However, we could not find any data on the toxic level of boron in the BD.

The effect of biogas digestate on the plant growth

The effects of treatments on plant dry weights, B, and other element concentrations of plants have been indicated in Table 5. The effects of individual factors of IP and BD dosages and their interactions significantly affectedplant dry weight (DW). Plant dry weights showed changes between 5.10-8.95 g and this variation showed significant differences with the application dosages ($P \leq 0.01$). Without BD applications (BD0) plant DW showed a significant variation with the incubation times. Similarly, IP resulted in an increase in DW under other BD dosages. Application of increased BD led to DW increase under all incubation periods. The DW showed 23% increase with the application of 30 t ha^{-1} BD. The most efficient IP on DW was found as 30 days. Boron concentrations in plants showed non-significant variations between 15.3 and 17 mg kg^{-1}. As in B, plant Cd and coconcentrations did not change significantly with individual factors and their interactions. While Cd concentrations varied between 0.05-0.08 mg kg^{-1}, Co concentrations changed between 0.06 to 0.09 mg kg^{-1}.

TABLE 1. Initial nutrient and heavy metal concentrations of the experimental soil.

Nutrients or elements in the soil	Concentrtion (mg kg^{-1})
Total N	800
Plant available P	5.4
Exchangeable K	298
Exchangeable Ca	6352
Exchangeable Mg	372
DTPA extractable Fe	1.5
DTPA extractable Zn	0.6
DTPA extractable Mn	5.3
DTPA extractable Cu	1.5
0.01 M $CaC1_2$ extractable B	0.44
Total Ni	85
Total Cr	44.8
Total Co	4.7
Total Cd	0.01
Total Pb	12.3

TABLE 2. Some characteristics of BD used for the experiment.

Total element concentrations (Dry matter basis)			
N (mg g^{-1})	18.6	Mn (mg kg^{-1})	254
C (mg g^{-1})	350	Cu (mg kg^{-1})	896
P (mg g^{-1})	9	B (mg kg^{-1})	55.0
Ca (mg g^{-1})	11.5	Cd (mg kg^{-1})	0.11
K (mg g^{-1})	36.7	Co (mg kg^{-1})	1.03
Mg (mg g^{-1})	10.7	Cr (mg kg^{-1})	6.45
Zn (mg kg^{-1})	192	Ni (mg kg^{-1})	13.8
Fe (mg kg^{-1})	3177	Pb (mg kg^{-1})	3.73

TABLE 3. Permissible levels of some heavy metals in organic residues used for agricultural purposes in different countries and organizations (Teglia et al., 2011).

Countries/ organizations	Elements (in dry matter, mg kg^{-1})						
	Zn	Cu	Cd	Co	Cr	Ni	Pb
RTMAF[1]	1100	450	3.0	-	350	120	150
British PAS[2]	400	200	1.5	-	100	50	200
CCME[3]	700	400	3.0	34	210	62	150
US EPA[4]	2800	1500	39	-	1200	420	300
NF U44-051[5]	600	300	3.0	-	120	60	180
RAL GZ251[6]	400	100	1.5	-	100	50	150

[1]: Republic of Turkey Ministry of Agriculture and Forestry (Regulation on organic, mineral and microbial source fertilizers used in agriculture). [2] British standards for biodegradable materials. [3]Canadian Council of the Ministers of the Environment (CCME: PN 1340 Guidelines for Compost Quality. [4]United States Environmental Protection Agency. [5]French standards for organic amendments (AFNOR: NF U44-051 Amendementsorganiques—De´nominations, spe´cifications et marquage. [6]: German standards for compost.

TABLE 4. Permissible levels of some heavy metals in the biosolids provided for some European Countries (Collivignarelli et al., 2019).

Countries	Elements (in dry matter, mg kg^{-1})					
	Zn	Cu	Cd	Cr	Ni	Pb
Germany	4000	800	10	900	200	900
Spain	4000	1750	40	1500	400	1200
France	3000	1000	20	1000	200	800
Italy	2500	1000	20	200	300	750
Netherlands	300	75	1.25	75	30	100
Denmark	4000	1000	0.8	100	30	120

TABLE 5. Effect of BD and IP on plant DW (g pot^{-1}) B and some heavy metal concentrations (mg kg^{-1}) of wheat.

Incubation (Days)	BD (t ha^{-1}) 0	15	30	Means
		DW		
0	5.10 e***	7.05 bc	6.55 cd	6.23 b**
30	7.17 bc	7.90 ab	7.83 ab	7.63 a
60	6.75 cd	5.67 de	8.95 a	7.12 ab
Means	6.34 B*	6.87 AB	7.78A	
		B		
0	16.6	15.3	15.4	15.8
30	16.2	15.9	16.5	16.2
60	16.1	16.7	17.0	16.6
Means	16.3	16.3	15.9	
		Cd		
0	0.08	0.07	0.05	0.08
30	0.07	0.07	0.07	0.07
60	0.07	0.07	0.07	0.07
Means	0.07	0.07	0.07	
		Co		
0	0.09	0.07	0.07	0.08
30	0.07	0.06	0.08	0.07
60	0.07	0.06	0.07	0.07
Means	0.08	0.06	0.07	
		Cr		
0	3.00	2.38	2.69	2.68
30	3.23	2.76	2.92	2.97
60	4.38	2.35	2.19	2.97
Means	3.52 A*	2.50 B	2.60 AB	
		Ni		
0	1.62 ab***	2.77 a	1.85 ab	2.08
30	2.16 ab	1.94 ab	2.09 ab	2.06
60	2.26 ab	1.37 b	1.35 b	1.66
Means	2.01	2.03	1.76	
		Pb		
0	0.52	0.43	0.33	0.43 a**
30	0.32	0.27	0.29	0.29 b
60	0.33	0.30	0.34	0.32 b
Means	0.39	0.34	0.32	

*: small letters shows the BD effect, **: capital letters shows the incubation effect, ***: small and superscribed letters shows the interaction effect.

Plant Cr concentrations were found to be between 2.19-4.38 mg kg-1. While the effect of incubation on these variations was not significant, the effect of BD dosages was significant. Plant Cr concentration under D0 conditions was 3.52 mg kg-1, this decreased to 2.50 and 2.60 mg kg-1 with applications of 1.5 and 3 t da-1 of BD. The effect of interaction on Ni concentrations was found to be significant. While the highest Ni concentration was found from the treatment of 15 t ha-1 BD under non-incubated conditions, the lowest was obtained from treatment of 30 t ha-1 BD under 60 days incubation. Depending on the treatments, Pb concentrations in wheat were determined between 1.644-3.053 mg kg-1. Plant Pb concentration significantly decreased with the incubation up to 33 %. Also, BD dosages resulted in decreases in plant Pb concentrations but these decreases were not significant. Boron and heavy metal uptakes of wheat are shown in Table 6. Looking at the B uptake, it was seen that all factor significantly affected plant B removal. Depending on the interaction the highest B uptake was obtained from combination of 30 t ha-1 BD and 60 days incubation whereas the lowest was obtained from the combination of 0 t ha-1 BD and 0 day incubation. If an evaluation was made on the individual factors, the most effective BD dosage was 15 t ha-1 and the most effective incubation day was 60 days. As for heavy metal uptakes of wheat, it has been observed that no factors had a significant effect on Co, Cr and Pb uptakes of wheat. When looked removed Cd amount by wheat, it can be seen that incubation periods had a significant effect onit. But when a comparision was made between the incubation days, only difference between 0 and 60 days incubation was significant on Cd uptake. Plant Pb uptakes showed significant variation with the interaction. The lowest Ni removal was measured from the control treatments of both individual factors, but the highest was obtained from the combination of 15 t ha-1 BD under 0 day incubation condition.

TABLE 6. Effect of BD and IP on B and some heavy metal uptakes (mg pot-1) of wheat.

Incubation (Days)	BD (t ha-1) 0	15	30	Means
		B		
0	84.66 c***	107.87 bc	100.87 bc	97.80 b**
30	116.15 abc	125.61 ab	129.20 bc	123.65 b
60	108.68 bc	94.69 bc	152.15 a	118.51 a
Means	103.16 B*	109.39 A	127.40 AB	
		Cd		
0	0.408	0.494	0.328	0.410 b*
30	0.502	0.553	0.548	0.534 ab
60	0.473	0.397	0.627	0.499 a
Means	0.461	0.481	0.501	
		Co		
0	0.459	0.494	0.459	0.471
30	0.502	0.474	0.626	0.534
60	0.473	0.340	0.627	0.480
Means	0.478	0.436	0.571	
		Cr		
0	15.30	16.78	17.62	16.57
30	23.16	21.80	22.86	20.61
60	29.57	13.32	19.60	20.83
Means	22.68	17.30	20.03	
		Ni		
0	8.26 b	19.53 a	12.12 ab	13.30
30	15.49 ab	15.33 ab	16.36 ab	15.73
60	15.26 ab	7.77 b	12.08 ab	11.70
Means	13.00	14.21	13.52	
		Pb		
0	2.65	3.03	2.16	2.61
30	2.29	2.13	2.27	2.23
60	2.23	1.70	3.04	2.32
Means	2.39	2.29	2.49	

*: small letters shows the BD effect, **: capital letters shows the incubation effect, ***: small and superscribed letters shows the interaction effect.

Discussion

Plant DW increased due to the increase in BD levels and incubation times compared to the control treatments. These results are in accordance with previous studies conducted with BD and different agricultural westes (El-Sebaey et al., 2005; Różyło et al., 2016; Sogn et al., 2018; Kara et al., 2019; Głowacka et al., 2020). It is thought that the increase in DW depending on the applications is closely related to the BD doses and incubations. The increase in the concentration of soil available nutrients due to BD application is one of the factors that increase plant growth. Also, the BD properties affects soil fertility which affects plant growth directly or indirectly (Barbosa et al., 2014; Abd-Eladl et al., 2016; Nabel et al., 2017; Walsh et al., 2018). Due to bioactive substances, such as vitamins, humic and fulvic acids, nucleic acids, free amino acids, monosaccharides, phytohormones, etc in it, digestat can promote plant growth, namely DW (Liu et al., 2009; Yu et al., 2010).

Plant B concentrations from each treatment were not affected by the treatments and it ranged between the sufficiency range (3-25 mg kg^{-1}) (Reuter & Robinson, 1997). In different recent studies conducted on different wheat genotypes under different growth environments, similar B levels were recorded as in our study (Turan et al., 2008; Rana et al., 2017; Fakir et al., 2018; Al-Ameri et al., 2019). Boron concentrations found in this study were close to those of Taban & Erdal (2000). They found that B concentration of above-ground part of eight weeks old 6 wheat genotypes varied between 10.0- 44.0 mg kg^{-1}. It was observed that most of the heavy metal concentrations of wheat were not affected by the applications, and some heavy metals were even adversely affected (Ni and Pb) by BD dosage and incubation treatments. This situation can be explained with the low B and heavy metal concentration of BD. As it was indicated before, our material has lower B and heavy metal concentrations than the permissible concentrations reported by different organizations (Teglia et al., 2011; Collivignarelli et al., 2019). An increase of soil buffering capacity due to BD dosages and incubation periods might be another result of the decreases in the availabilities of the elements. Also, soil heavy metal concentrationsare quite below the world standards indicated for agricultural soils (Li et al., 2009; Zhou et al., 2014). Concentrations of Ni found in the present study were quite lower than those measured in previous studies (Bose & Bhattacharyya, 2008; Wang et al., 2015). The wheat Cd concentration we obtained is well below the wheat Cd concentrations previously determined by Köleli et al. (2004) and by Cui et al. (2012). According to the previous records, critical Cd levels that can create oxidative stress on wheat seedlings were between 3.3 and 10 mg kg^{-1} (Bingham et al., 1975). Hara & Sonoda (1979) pointed out the critical Cr concentration as 10 mg kg^{-1} in barley. Gedikoğlu et al. (1998) recorded the toxic concentration of Co as 20-25 mg kg^{-1} in some cereals such as barley. In this study, it was determined that heavy metal concentrations of wheat were found to be below the toxicity levels indicated by different researchers (Mamata et al., 2009; Yurdakul et al., 2017). In this study, it can be conducted that element uptake of wheat applied did not increase with BD dosages and incubation periods. Element removals were closely related to plant DW mostly.

Looking at the amount of potentially toxic elements in digestate used in this research are quite below the threshold levels given by different countries and organizations for organic wastes and biosolids used for agricultural purposes (Teglia et al., 2011; Collivignarelli, 2019). Digestate used in this study also has lower heavy metal concentrations than permissible concentrations given by Republic of Turkey, Ministry of Agriculture and Forestry. So it is suitable for using agricultural purposes. Similarlly, Odlare et al. (2008) did not find any negative effects on the soil. As it can be observed that the limit values indicated differ between countries.

Conclusion

BD dosages resulted in an increase in DW under each incubation period. Looking at the mean values, plant DW increased up to 23% with the application of 30 t ha^{-1} and 22% with 30 days incubation. Looked at the increasing tendency in DW, it can be concluded that DW may increase if higher doses of BD applied. But as for IP, the most suitable incubation period is 30 days. Looking at the variations of potentially toxic element concentrations in the plant, there is not a potential of BD to create toxicity on plant growth under incubated or non-incubated conditions. For this reasons, BD's having the similar properties we used, can be used as soil amender or fertilizer source when used at the rate used in this study. Thus, on the one hand, BD, which is an important problem to be disposed of, will be evaluated in an environmentally friendly manner, and on the other hand, thanks to the contributions of BD to plant development, the use of chemical fertilizers will be saved.

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Author contribution

All outhors have equal contributionon manuscript. They have read and agreed to the version of the manuscript.

Consent for publication

All authors declare their consent for publication.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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