

BIOGAS FACILITY-BASED ORGANIC FERTILIZER ENHANCES NUTRIENT UPTAKE AND GROWTH OF WHEAT (*TRITICUM AESTIVUM*)

ERENOĞLU, E. B.* – HACİRÜSTEMOĞLU, K. D.

*Çukurova University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition
Balcalı-Sarıçam, 01330 Adana, Türkiye*

*Corresponding author
e-mail: ebulenterenoglu@cukurova.edu.tr

(Received 26th Jul 2022; accepted 20th Oct 2022)

Abstract. The present study aimed to evaluate the effectiveness of organomineral fertilizers, which have become widespread in recent years. For this purpose, a pot experiment under greenhouse conditions was carried out to show the effect of nitrogen (N), phosphorus (P), and potassium (K) applied together or separately with an organic fertilizer (OF) originating from the biogas plant on the growth and the nutrient uptake of the model plant wheat (*Triticum aestivum* L. cv. Adana-99). Two different fertilizer application methods (incorporating and placement) were mimicked, and the efficacy of biogas plant-derived organic fertilizers was determined. The results of the study show that the OF applied with the plant nutrients positively affected plant growth and nutrient uptake. According to the correlation analysis, significant correlations were found between the OF application and the shoot dry weights of the plants and their P uptake. The fertilizer application 5 cm below the seedbed caused the most effective results. The correlative relationships detected in plants where the OF and N, P, and K were applied together disappeared when plant nutrients were mixed to the entire pot, and OF was used 5-10 cm or 5 cm below the seedbed. The results showed that biogas facility-based OF applications, together with the plant nutrients in the form of an organomineral fertilizer, can increase the nutrient use efficiency of plants by improving their uptake, especially that of P.

Keywords: *humic substances, phosphorus, organomineral, uptake, use efficiency*

Introduction

The increasing total population, the declining rural population, increases in income levels, and changes in the rate of poverty in the population over time constitute the main socio-economic factors that lead to increased food demand at present and in the future (FAO, 2009; Msangi and Rosegrant, 2009). Moreover, this increase must be achieved on already limited agricultural production lands which cannot be increased further. In addition, agricultural areas are also faced with losing fertility/productivity due to water constraints, mismanagement activities, and the improper and imbalanced use of agricultural chemicals. The global population growth increases the demand for agricultural production leading to a rising dependence on fertilizer inputs (Kominko et al., 2017) which require energy for their production. For this reason, both increasing plant yield and minimizing possible environmental risks due to plant nutrients are among the primary goals of today's plant nutrition activities.

Although mineral nutrient sources have been used in plant production since the last century, organic sources, including animal manure, have been widely applied to serve as essential nutrients in crop production. Organic fertilizers can provide organic matter when applied to the soil. When used effectively, they can provide conditions for crop production that exceed those ensured by mineral fertilizers (MFs) alone (Steiner et al., 2007; Ayinla et al., 2018). Moreover, since organic wastes contain various nutrients, their use can

reduce the need for mineral fertilizers; this is particularly important in regard to declining mineral fertilizer raw materials e.g., of phosphorus (Adewopo et al., 2014; Amundson et al., 2015). Although manure is extensively used in agriculture, there are several known handicaps to its use. For example, to meet crop nutritional needs, MFs must often be used to balance the nutrients in manure, which has low nitrogen (N) to phosphorus (P) ratio (Smith et al., 2020). Application of manure to meet crops' N needs will, in most cases, result in an over-application of P, leading to environmental issues over time. Combining MFs with manure or other waste products such as sewage sludge and biogas digestates through industrial processes to produce a new product, organomineral fertilizers (OMFs), is one promising alternative (Smith et al., 2020).

In recent years, biogas digestates have been used as organic fertilizing materials in plant production. Power and heat generation from biogas significantly contribute to bioenergy production (Ehmann et al., 2018). Moreover, biogas digestates as OF or organic raw materials for OMFs seem to be an effective method of closing nutrient cycles in agriculture and reducing external inputs of MF.

In general, soil organic matter has many positive effects on the physical, chemical, and biological properties of the soil because of complex relationships, as well as a triggering effect on the growth and development of plants (Baldotto et al., 2012; Zandonadi and Busato, 2012; Zandonadi et al., 2013; Canellas and Olivares, 2014). Organic matter application to the soil promotes the decomposition of substances and establishes microbial equilibrium (Kominko et al., 2017). In addition to its positive effects on soil properties, it directly affects plant physiology and growth, especially in improving root development (Canellas and Olivares, 2014).

Humic substances constitute 80% of the soil organic matter (Brady and Weil, 2017). The most common elements in humic substances are carbon (C), oxygen (O), hydrogen (H), N, and sulfur (S) (Stelink, 2002). There are three classifications of humic substances based on their solubility: fulvic acid, humic acid, and humin (Lag et al., 2008). From a biological perspective, humic substances increase soil fertility by affecting the composition of microbial populations (Calvo et al., 2014). In parallel with this, a study conducted in the calcareous soils of the Mediterranean Region showed that the application of humic-fulvic acid plays a significant role in the recovery of P in the soil (Delgado et al., 2002). Likewise, Tahir et al. (2011) stated that humic acid application increased the P content of wheat grown in calcareous soils.

The positive effects of humic substances in the structure of organic materials on the development of many plants have been reported frequently (Visser, 1986; Chen and Aviad, 1990). In many studies, it has been found that humic substances increase nutrient uptake due to the increase in the permeability of cell membranes; it has been shown that they have an auxin hormone-like effect with chelation and transport of nutrients and reduce the impact of damage caused by abiotic stresses (Garcia et al., 2012a, b). Many researchers have demonstrated that adding humic substances at certain levels can improve both root and stem growth and promote the uptake of nutrients as well. It has been shown in a recent study that the application of one ton of vermicompost per hectare in addition to adequate NPK rates in sweet corn, increased the biomass, grain yield, and the total N, P, and K uptakes (Canatoy and Daquiado, 2021). However, in another study under greenhouse conditions conducted using wheat such a good relationship between OF application, biomass development, and N, P, and K uptakes could not be shown (Mumbach et al., 2018).

There are still points to be explored about the contribution of organic resources to plant production and the mechanisms that play a role in this process. Up to recent years, OFs and MFs were applied separately under field conditions, however, the production and use of granulated OMFs which are a good mixture of OFs and MFs are increasing worldwide. Most likely, the effects of OFs and MFs when applied together will differ from their effects when applied separately, as spatial availability will be affected. With these considerations, the current research project was planned to contribute to the knowledge about the possible effects and tools. With this aim, two pot experiments were designed using wheat as a model plant in greenhouse conditions. In the first part of the study, to test the effect on the growth and nutrient uptake of the model plant wheat, increasing rates of OF with fixed rates of N, P, and K containing MFs were applied to specific sites of pots (whole pot, 5-10 cm below seedbed, and 5 cm below seedbed). In the second part, to reveal the possible role of the OF implementation independent of MF sources, the same rates of OF were applied to 5-10 cm below seedbed and 5 cm below seedbed. In contrast, the mineral nutrient sources were applied to the whole pot in the same fixed quantities as in the first part.

Materials and Methods

Materials

The study was conducted in the experimental greenhouses of Soil Science and Plant Nutrition Department at Çukurova University in Adana, Türkiye. A spring bread wheat (*Triticum aestivum* L.) variety, Adana-99, extensively cultivated in the region, was evaluated in the pot experiment conducted under greenhouse conditions. The pots used in the experiment were cylindrical and obtained from PVC pipes with a diameter (\emptyset) of 12.5 cm. The pipes were cut in 24 cm depths, and their bottoms were covered with a PVC material with holes. The Arik Series soil with low organic matter content (1.17%), high pH (7.63), calcium carbonate (27.2%), and clay content (54.8%) was used to represent most soils of the Çukurova Region and Turkey. The soil taken from the field was air-dried and sieved (4 mm) before being used in the experiment.

The organic fertilizer source used in the study was a composted granular material obtained from biogas facility, containing 53% organic matter, 20% humic + fulvic acid, 1.98% nitrogen (N), 3.02% phosphorus (P_2O_5), and 0.91% potassium (K_2O), with a C/N ratio of 15.6 and used after grinding.

Effect of Organic Fertilizer Applied to the Same Place with Mineral Fertilizers on Wheat - Experiment I

The experiment was conducted using four different OF rates (0, 100, 200, and 400 mg $OF \cdot kg^{-1}$ soil or 0, 0.25, 0.50, and 1 ton $OF \cdot ha^{-1}$) and three different fertilizer placements (*Figure 1; Table 1*). The organic fertilizer rates were determined to include the 0.50-0.75 ton $OF \cdot ha^{-1}$ of the fertilizer recommended by the producer for wheat cultivation. Nitrogen, P, and K fertilizers were urea, triple superphosphate (TSP), and potassium sulfate (KS). The MF sources were used after grinding as with OF. Nitrogen, P, and K rates were 200 mg $N \cdot kg^{-1}$ soil, 100 mg $P \cdot kg^{-1}$ soil, and 100 mg $K \cdot kg^{-1}$ soil (*Table 1*). The organic fertilizer, together with inorganic nutrient sources, was placed in three distinct positions of 24 cm-high pots (the soil height was 20 cm) as whole pot, 5-10 cm below seedbed, and 5 cm below seedbed (*Table 1*). For each treatment, three pots

(replicates) were used and each pot was filled with 2.5 kg of soil. After the placement of organic and mineral fertilizer sources, twelve seeds were sown, and the seedlings were thinned to six plants per pot discarding the excess ones. After planting, the pots were regularly weighed and watered at 50% of the field capacity for the first week and 75% for the ongoing weeks.

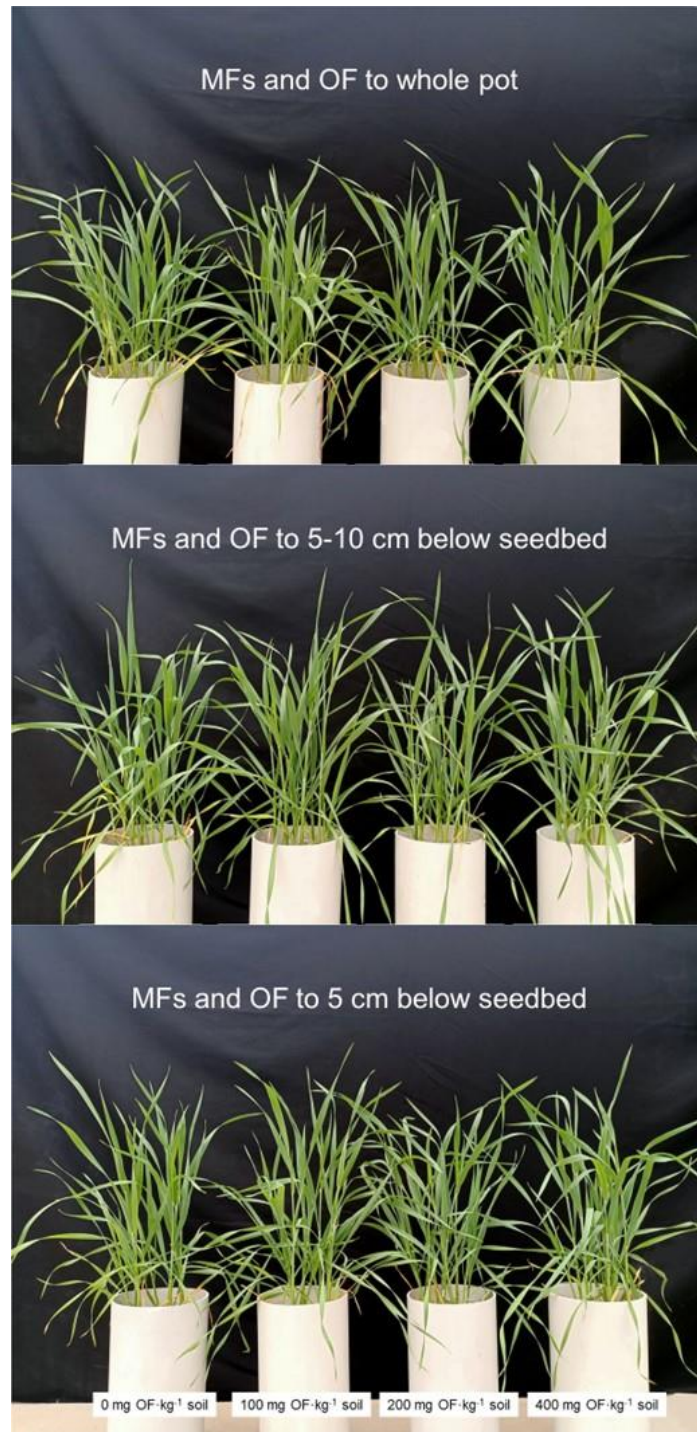


Figure 1. A view from Experiment I in which wheat plants grown with increasing OF rates placed at different sites (whole pot, 5-10 cm below the seedbed and 5 cm below the seedbed) together with N, P, and K in pots for 45 days under greenhouse conditions

Table 1. Experimental variables and nutrient rates used in Experiment I and Experiment II

Nutrient / Soil Amendment	Source	Amounts of Nutrient / Soil Amendment		Placement
		mg·kg ⁻¹ soil	mg·pot ⁻¹	
Organic Fertilizer*	Biogas Facility-Based	0	0	<p>Experiment I</p> <ul style="list-style-type: none"> organic and chemical fertilizers to the whole pot organic and chemical fertilizers to 5-10 cm below seedbed organic and chemical fertilizers to 5 cm below seedbed <p>Experiment II</p> <ul style="list-style-type: none"> chemical fertilizer to the whole pot (0 mg OF·kg⁻¹ soil) organic fertilizer to 5-10 cm below seedbed, chemical fertilizers to the whole pot organic fertilizer to 5 cm below seedbed, chemical fertilizers to the whole pot
		100	250	
		200	500	
		400	1000	
N	Urea	200	500	
P	TSP	100	250	
K	K ₂ SO ₄	100	250	

* The organic fertilizer rates (0, 0.25, 0.50, and 1.00 ton OF·ha⁻¹) were determined to include the rates, 0.50-0.75 ton OF·ha⁻¹, of the fertilizer recommended by the producer for wheat

At the end of the 45-day experimental period, the shoots were harvested, dried at 65 °C, weighed, and dry ashed; then K, Ca, and Mg contents were determined using Atomic Absorption Spectroscopy. Total N content in shoots was measured using the Kjeldahl method. Phosphorus concentration in shoots was measured using UV visible spectrophotometry.

Effect of Organic Fertilizer Applied Separately with Mineral Fertilizers on Wheat - Experiment II

In this experiment, the OF rates, mineral fertilizer sources and rates, and all experimental procedures from sowing to mineral element analyses were as given for *Experiment I*; however, fertilizer application places differed (*Table 1*). In such a way, while all chemical sources were mixed in a whole pot, organic fertilizer was placed in distinct positions, as i) organic fertilizer to 5-10 cm below seedbed, chemical fertilizers to the whole pot, and ii) organic fertilizer to 5 cm below seedbed, chemical fertilizers to the whole pot. Moreover, the MF sources of plants grown without OF (0 mg OF·kg⁻¹ soil) were mixed in the whole pot.

Statistics

For the first experiment, where organic and inorganic fertilizers were placed together but at different points of the pot, a two-way analysis of variance was performed using SPSS, considering four different OF rates and three different application sites. In addition, each placement was regarded as a separate group and subjected to the one-way ANOVA. For the second experiment, where chemical fertilizers are applied to the whole pot, and increasing rates of the OF were applied to 5-10 cm or 5 cm below the seedbed, OF was subjected to a one-way analysis of variance with SPSS. In both experiments, the Duncan test ($p < .05$) was used to measure specific differences between pairs of means.

Results

Experiment I

In this experiment, the bread wheat cv. Adana-99 was grown in pots where OF was placed at increasing rates and with mineral fertilizer sources containing N, P, and K, in different sites of pots. The application 5 cm below the seedbed caused the lowest shoot growth (*Figure 2A*). There was no difference between mixing the whole pot and mixing 5-10 cm below the surface. Although increasing rates of the OF applied as mixing 5-10 cm below the seedbed had a positive effect on the shoot growth of wheat, this effect was statistically insignificant (*Figure 2A, B*). On the contrary, mixing the whole pot and 5 cm below the seedbed applications caused statistically significant expansions in the growth of the shoot as a response to increasing rates of OF (*Figure 2A, B*). However, the most significant rise in the shoot growth was obtained when the OF and MFs were applied 5 cm below the surface (*Figure 2B*). Although the highest shoot growths were achieved at 400 mg OF·kg⁻¹ soil in both fertilizer placements, it did not cause statistically apparent differences compared to 100 and 200 mg OF·kg⁻¹ soil. While there was a statistically significant increase in 200 mg OF·kg⁻¹ soil compared to 0 mg OF·kg⁻¹ soil in pots where the OF was mixed the whole pot together with MFs, 100 mg OF·kg⁻¹ soil has come to fore as the sufficient rate in applications 5 cm below the seedbed.

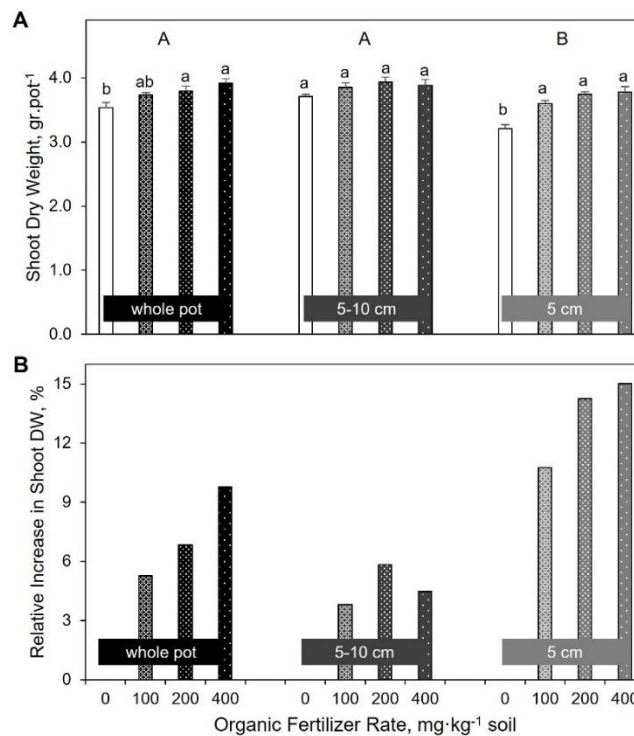


Figure 2. Shoot dry weights (A) and relative dry weight increases (B) relative to 0 mg OF·kg⁻¹ soil of wheat plants grown with increasing OF rates placed at different sites (whole pot, 5-10 cm below the seedbed and 5 cm below the seedbed) together with N, P, and K in pots for 45 days under greenhouse conditions. The dry weight columns are the mean of three independent replications \pm SE. According to ANOVA and Duncan, the lower cases represent the differences at each application site's $p < .05$ level. Capital letters indicate differences at the $p < .05$ level between application sites according to the two-way ANOVA and Duncan, where application sites and organic fertilizer rates are variable. The columns for relative dry weight increases are calculated from dry weight averages

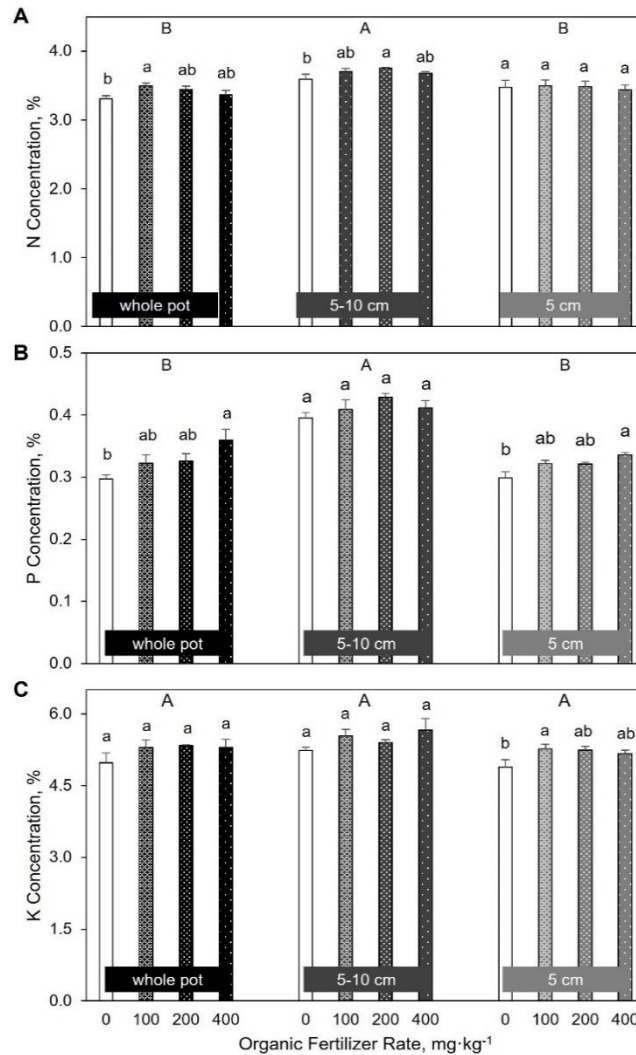


Figure 3. Nitrogen (A), P (B), and K (C) concentrations in shoots of wheat plants grown with increasing OF rates placed at different sites (whole pot, 5-10 cm below the seedbed and 5 cm below the seedbed) together with N, P, and K in pots for 45 days under greenhouse conditions.

Concentration columns are the mean of three independent replications \pm SE. According to ANOVA and Duncan, the lower cases on columns represent the differences at each application site's $p < .05$ level. Capital letters indicate differences at the $p < .05$ level between application sites according to the two-way ANOVA and Duncan, where application sites and organic fertilizer rates are variable

As seen in Figures 3A and C, increasing rates of the OF applied together with mineral N, P, and K sources to various places did not have a statistically significant effect on the shoot N and K concentrations. However, when the application sites were considered, it was observed that the highest average N concentration values were obtained when the OF and MFs were applied 5-10 cm below the seedbed. As given in Figure 3B, increasing OF rates had statistically meaningful effects on the P concentrations of the shoot. In the treatments where the OF and MF sources were applied to the whole pot and 5 cm below the seedbed, the OF significantly increased the P concentration in the shoot compared to 0 mg OF·kg⁻¹-used plants. When the OF and MFs were mixed 5-10 cm below the seedbed, increasing amounts of OF did not affect the P concentration of the shoot. As in the case

of N, the highest average of P concentration values were obtained when the OF and MFs were applied 5-10 cm below the seedbed. As can be seen in the results presented in *Figure 3C*, increasing OF rates did not have any effect on the shoot K concentrations of the wheat plant. There was no difference between the mean values of the K concentrations at different OF rates measured in the whole pot, 5-10 cm below the seedbed, and 5 cm below the seedbed.

Figures 4A, B, and C demonstrate the N, P, and K uptake values per pot obtained by multiplying the concentrations of each nutrient in the shoot tissues of the wheat plant grown with the increasing rates of OF applied together with the mineral N, P, and K sources, in different sites of the pots, respectively. Considering the application sites, the highest N uptake values per pot were obtained in the case of all fertilizers applied 5-10 cm below the seedbed (*Figure 4A*). Compared to pots without OF application, N uptake per pot increased at all three OF rates, but there was no statistically significant difference among them. As for N, the highest P uptake values per pot were obtained with applications 5-10 cm below the seedbed (*Figure 4B*). Mixing to the whole pot and increasing the OF boosted P uptake per pot in all three application sites (*Figure 4B*). Still, the results were statistically more significant when the OF and MF sources were placed in the entire pot, particularly 5 cm below the seedbed (*Figure 4B*). When application sites are considered, the highest K uptake per pot was calculated in plants grown with the OF and MFs applied 5-10 cm below the seedbed; this was followed by the application mixed to the whole pot (*Figure 4C*). However, increasing the OF enhanced K uptake in all three application sites.

The results of the correlation analysis showing the relationship between the applied OF rates, shoot dry weights, and the N, P, and K concentrations in the shoot are given in *Table 2*. As can be seen, no statistically significant relationship was observed between shoot dry weights, N, P, and K concentrations obtained due to the applications the OF and N, P, and K-containing fertilizers in the form of mixing to 5-10 cm below the seedbed. Only N and P concentrations were correlated at the .01 level. A significant correlation (.01) was found between the OF rates and shoot dry weights, P concentrations in whole pot mixing, and 5 cm below the seedbed applications. There was no positive correlation between the OF rate and shoot N and K concentrations in those treatments.

Experiment II

In the second part of the study, the wheat cv. Adana-99 was grown for 45 days in pots where the OF was applied to 5-10 cm and 5 cm below the seedbed, but MFs were placed separately from the OF and mixed with the entire soil. The results showed that the OF set 5-10 cm below the seedbed generally caused better shoot growth than the one applied 5 cm below the seedbed (*Figure 5A*). While in the case of the OF mixed 5-10 cm below the seedbed, 200 mg OF·kg⁻¹ soil rate produced better shoot growth than that of the control (0 mg OF·kg⁻¹ soil), the shoot growth was better in the 400 mg OF·kg⁻¹ soil for 5 cm below the seedbed applications. *Figure 5B* shows the relative increases in shoot dry weights obtained with OF applications, expressing the ratio of the average shoot dry weight of the control application in which no OF was added and MFs were mixed with the whole pot to the shoot dry weights of other treatments. The highest increase was achieved in the OF mixed 5-10 cm below the seedbed. Consistent with their dry weights, no difference was observed between 200 and 400 mg OF·kg⁻¹ soil rates. Among the OFs applied 5 cm below the seedbed, the highest increase was obtained at the rate of 400 mg OF·kg⁻¹ soil.

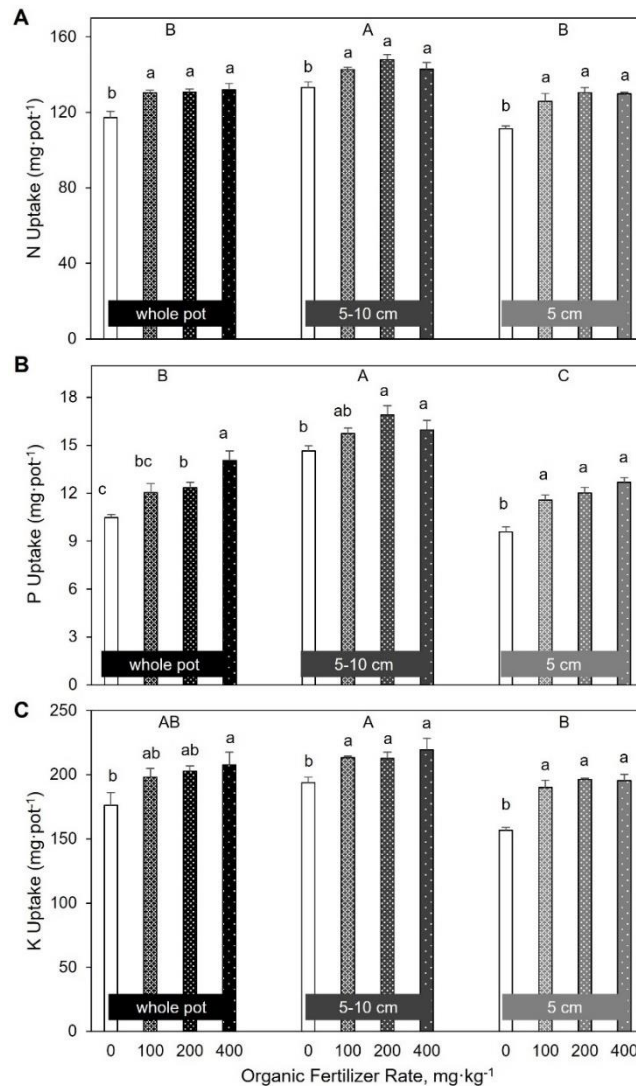


Figure 4. Nitrogen (A), P (B), and K (C) uptakes of wheat plants grown with increasing OF rates placed at different sites (whole pot, 5-10 cm below the seedbed and 5 cm below the seedbed) together with N, P, and K in pots for 45 days under greenhouse conditions. Uptake columns are the mean of three independent replications \pm SE. According to ANOVA and Duncan, the lower cases on columns represent the differences at each application site's $p < .05$ level. Capital letters indicate differences at the $p < .05$ level between application sites according to the two-way ANOVA and Duncan, where application sites and organic fertilizer rates are variable

Figures 6A, B, and C show N, P, and K concentrations in the shoot of wheat grown for 45 days in pots where MFs were applied to the whole pot and where varying rates of the OF were positioned to different sites, respectively. As can be seen, in general, no correlation was observed between the N concentrations in the shoot and the OF application rates or sites (Figure 6A). Like N concentrations, the P and K concentrations in the shoot and the OF rates or locations did not demonstrate any correlation (Figures 6B and C, respectively). In contrast to Experiment I, there was no difference between the P concentrations in shoots of wheat plants grown without the OF and those of plants grown with the highest OF rate.

Table 2. Correlation between organic fertilizer rates (OFR) and shoot dry weight (SDW) and N, P, and K concentrations in the wheat supplied with increasing rates of OF placed at different sites (whole pot, 5-10 cm below the seedbed and 5 cm below the seedbed) together with N, P and K in pots for 45 days under greenhouse conditions

Placement	Factor	OFR	SDW	N	P	K
whole pot	OFR	-	-	-	-	-
	SDW	0,813**	-	-	-	-
	N	0,107	0,114	-	-	-
	P	0,721**	0,156	0,164	-	-
	K	0,426	0,645*	0,225	0,396	-
5-10 cm	OFR	-	-	-	-	-
	SDW	0,502	-	-	-	-
	N	0,411	0,281	-	-	-
	P	0,388	0,331	0,724**	-	-
	K	0,497	0,019	0,108	0,481	-
5 cm	OFR	-	-	-	-	-
	SDW	0,854**	-	-	-	-
	N	-0,110	-0,239	-	-	-
	P	0,781**	0,643*	0,005	-	-
	K	0,435	0,490	0,578*	0,482	-

** Correlation is significant at a 0.01 level, *. Correlation is significant at a 0.05 level

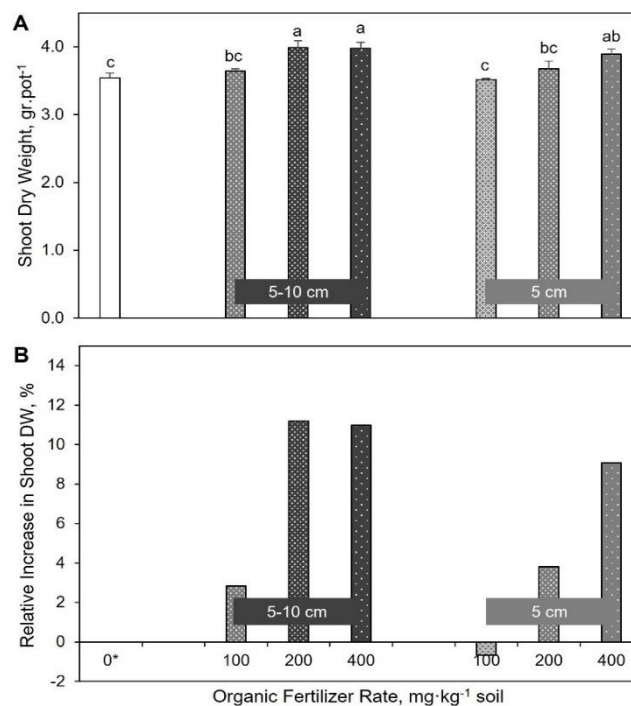


Figure 5. Shoot dry weights (A) and relative dry weight increases (B) relative to 0 mg OF.kg⁻¹ soil of wheat plants grown with increasing OF rates placed at different sites (5-10 cm below the seedbed and 5 cm below the seedbed) separately from N, P, and K (whole pot) in pots for 45 days under greenhouse conditions. The dry weight columns are the mean of three independent replications \pm SE. According to ANOVA and Duncan, the lower cases represent the differences at each application site's $p < .05$ level. Capital letters indicate differences at the $p < .05$ level between application sites according to the two-way ANOVA and Duncan, where application sites and organic fertilizer rates are variable. The columns for relative dry weight increases are calculated from dry weight averages

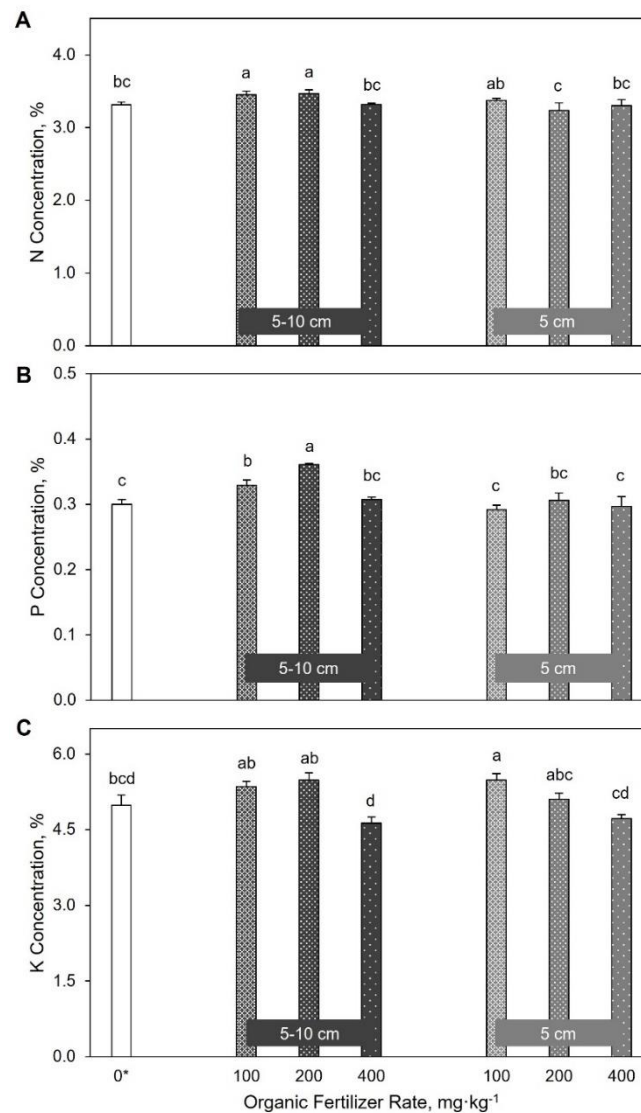


Figure 6. Nitrogen (A), P (B), and K (C) concentrations in shoots of wheat plants grown with increasing OF rates placed at different sites (whole pot, 5-10 cm below the seedbed and 5 cm below the seedbed) separately from N, P, and K (whole pot) in pots for 45 days under greenhouse conditions. Concentration columns are the mean of three independent replications \pm SE. According to ANOVA and Duncan, the lower cases on columns represent the differences at each application site's $p < .05$ level. Capital letters indicate differences at the $p < .05$ level between application sites according to the two-way ANOVA and Duncan, where application sites and organic fertilizer rates are variable

Figures 7A, B, and C represent the effects of the increasing rates of OF, which is mixed 5-10 cm below the surface or applied to the tape 5 cm below the surface, on the N, P, and K uptakes per pot, respectively. Organic fertilizer application either did not increase the N uptake of plants compared to the control pot or caused changes unrelated to the applied rates (Figure 7A). The N uptake values in the pots where the organic fertilizer was mixed 5-10 cm below the seedbed were slightly higher than those applied to the 5 cm below the seedbed. Considering the application sites, the highest P uptake values per pot were calculated when the OF was applied 5-10 cm below the seedbed (Figure 7B). There was

no statistically significant difference between the P uptake values of the plants in the pots where the OF was applied 5 cm below the surface and the P uptake values of the control plants. When the application sites are taken into consideration, the K uptake values of the plants did not differ (Figure 7C). In addition, there was no relationship between the OF rates and the K uptake of wheat.

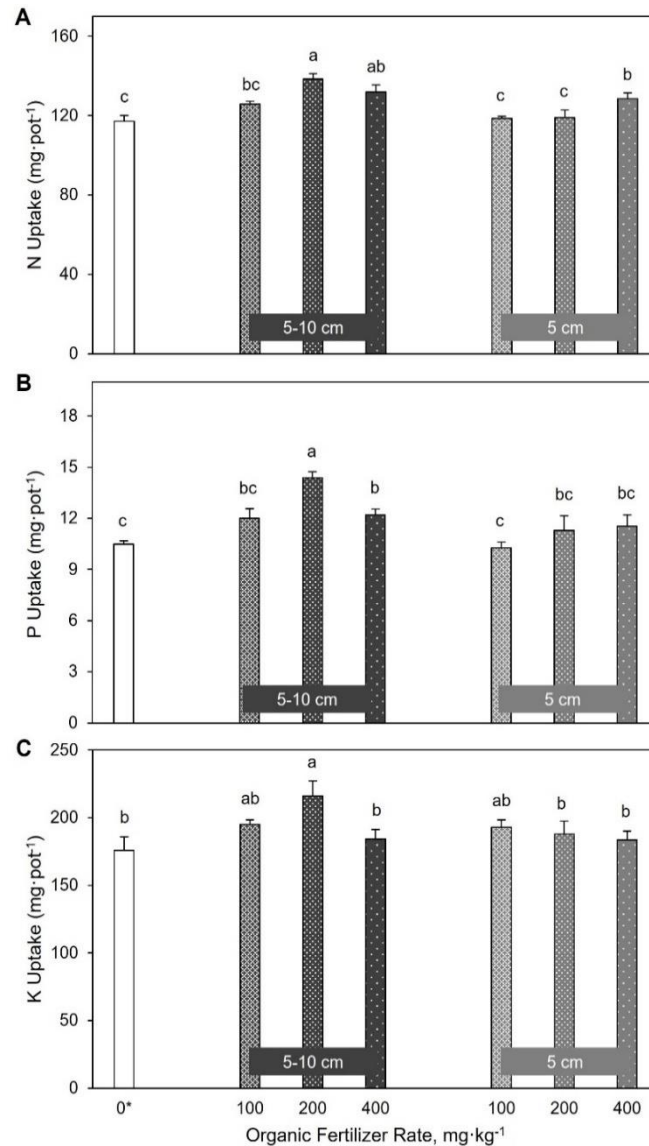


Figure 7. Nitrogen (A), P (B), and K (C) uptakes of wheat plants grown with increasing OF rates placed at different sites (whole pot, 5-10 cm below the seedbed and 5 cm below the seedbed) separately from N, P, and K (whole pot) in pots for 45 days under greenhouse conditions. Uptake columns are the mean of three independent replications \pm SE. According to ANOVA and Duncan, the lower cases on columns represent the differences at each application site's $p < .05$ level. Capital letters indicate differences at the $p < .05$ level between application sites according to the two-way ANOVA and Duncan, where application sites and organic fertilizer rates are variable

Discussion

An effective system for recycling agricultural, urban, and industrial organic wastes under field conditions can offer a more sustainable solution to organic waste problems while maintaining soil fertility. Bringing the leftover of products from urban wastewater sludge, agricultural industry activities, and biogas plants to agricultural production areas can also be part of such activities. In recent years, many studies have foregrounded the significance of organic matter for climate change (Amundson et al., 2015; Baveye, 2015; Eden et al., 2017). Moreover, Baveye (2015) emphasized the need for modified agricultural practices to improve water and nutrient retention in the cropped soil layer among the grand challenges in research on soil-plant systems. Thus, it is essential to scientifically evaluate the application of wastes originating from biogas plants, the use of which has increased significantly in recent years in agricultural areas.

As mentioned earlier, humic substances constitute 80% of soil organic matter (Brady and Weil, 2017) and can be operationally classified into three separate fractions defined in terms of their solubility properties (Lag et al., 2008). From a biological perspective, humic substances increase soil fertility by affecting the composition of microbial populations (Calvo et al., 2014). Organic fertilizers and humic substances, which are the main components of soil organic matter and are beneficial in maintaining the sustainability and productivity of soils besides disposing of waste, also indirectly affect the growth/yield and nutrition of plants (Eden et al., 2017).

Plant Growth

According to the results of the present research, the biogas plant residue-based OF application in the range used by farmers in practice significantly affect the shoot growth and nutrient uptake of wheat. The results obtained align with the review article by Smith et al. (2020) and the research article published by Shafi et al. (2020). In this respect, the results of the present research work show the positive effect of the OF application on plant growth when it is applied together with nutrients (*Figure 2*) and, to some extent, separately from them (*Figure 5*). The applications of single superphosphate and the organic amendment in the form of HA at increasing rates improve the agronomic responses of wheat under field conditions (Shafi et al., 2020). However, in a study conducted in England using wastewater treatment sludge, OMFs obtained using wastewater treatment sludge resulted in higher grain yields than the direct use of the OF and lower grain yields than urea (Antille et al., 2017). In parallel, Ehmann et al. (2018) stated that the best results are obtained from mineral fertilizers compared to biogas plant digestate, considering the yield parameters of silage maize. However, the OM content of the soils in the two fields where those studies were conducted varied between 4.6% and 7.9%, and the OF rate was 17 tons·ha⁻¹. The organic matter of the soil used in the current study was 1.17%, and the highest application rate was one-ton·ha⁻¹. In another study under greenhouse conditions, Mumbach et al. (2019) also did not observe any effect of fertilizer application in the organomineral form (mineral fertilizer + broiler litter) on plant growth and nutrient uptake. The reason for this condition might be the low OF rate used in the experiment compared to usual practice. These conflicting results show that the benefit obtained from the biogas facility-based OF (or any other organic material) may be closely related to the application rate of the OF, the humic substances in the OF, the application methods of the OF (together with nutrients or separately from them), and the OM content of the soil. While the application of the OF together with N, P, and K to the

whole pot (broadcasting) and 5 cm below the seedbed (banding) increases the shoot growth of plants, this increment is more evident in the application to 5 cm below the seedbed (*Figure 2A, B*). Implementations to the 5-10 cm below the seedbed were not effective. This result also shows the correlation between the OF and shoot growth. In other words, the correlations between OF rates and dry weight vary as 5 cm below the seedbed > whole pot > 5-10 cm below the seedbed depending on the application site (*Table 2*). It was observed that the combined applications of the OF and N, P, and K sources as mixing to the 5-10 cm below the seedbed and whole pot mixing, regardless of the OF rate, caused the highest average shoot dry weight value (*Figure 2A*). This indicates that the plants are better fed in applications 5-10 cm below the seedbed. This situation is also demonstrated by the plants' higher N and P concentrations compared to other treatments (*Figure 3A, B*) and the N, P, and K uptake per pot (*Figure 4A, B, C*) of the application in question. This is a good indicator of the response of plants to the nutritional elements applied through fertilizers (Herencia and Maqueda, 2016). However, it is not the case in field treatments where granular fertilizers are not mixed to a specific soil layer; they are incorporated into the soil after broadcasting (whole pot mixing) or banded below the seedbed (5 cm below the seedbed placement).

Nutrient Uptake

In line with the results obtained in sweet corn by Canatoy and Daquiado (2021), the results obtained from the study show that N, P and K uptakes are all increased relative to the control with the OF addition, especially when OF and MFs are applied together (*Figure 4*). Between the OF that is applied together with N, P, and K, placement and the nutrient concentrations in the shoot, the most meaningful relation exists for P. In other words, in contrast to the 5-10 cm below seedbed placements and N (*Figure 3A*) and K (*Figure 3C*), linear relations are observable between P concentrations and OF rates in the whole pot mixing and the 5 cm below seedbed placement (*Figure 3B*). Irrespective of placement, the OF application increases the P uptake of wheat (*Figure 4B*). The effect in question is similar to the results of Shafi et al. (2020) in wheat under field conditions. In such a way, the organic amendment applied as HA at a rate of 5 kg ha⁻¹ increases the leaf P concentration and total P uptake. However, the present study's minimum HA + FA rate is almost 50 kg ha⁻¹. These findings are in good accordance with the results of a previous study in which the total N and P removed from the unit area were evaluated (Sharif et al., 2013), and with the results of the current study, which determined that N (*Figure 4A*), P (*Figure 4B*), and K (*Figure 4C*) uptakes per pot increased irrespective of the OF rates. In addition, a correlation exists between the OF rates and P concentration of shoots when the OF is mixed with mineral fertilizer sources in the whole pot and placed 5 cm below the seedbed (*Table 2*). A recent study showed that compost enriched with phosphate rock used before planting increased nodulation, yield, and P uptake (both in concentration and per unit area) in chickpeas (Ditta et al., 2018).

These results show that OFs and especially HA + FA in its content form P soluble complexes, promoting plant growth (Nardi et al., 2000), and providing plants with an excellent physicochemical and biological environment in the soil (Brannon and Summers, 1985) may have increased P availability. In addition, the close relationship between shoot growth (*Figure 2*), P uptake (*Figure 4B*), and the OF is shown in *Table 2*, revealing that the application of organic amendment rich in humin substances with P can lead to increases in yield. The increase in growth may be related to the assimilation of macro and micronutrients, enzyme activation and inhibition, changes in membrane permeability,

protein synthesis that increases the biomass, or the stimulatory effect of P and HA on plant growth or biological production (Datta et al., 2017a, b). The high P concentration measured in plant leaves because of the application of the OF, and indirectly the HA + FA contained in it with P is consistent with the previous results of researchers (Cooper et al., 1998; Atiyeh et al., 2002). They stated that adding HA to the soil increased root growth, proliferation, branching, and initiation of root hairs, thus enabling the roots to capture more nutrients, and increasing nutrient concentrations in plants. Many studies have shown an increase in root length, root number, and root branching with HA application. However, the increase in root growth is usually more pronounced than shoot growth (Erdal et al., 2000).

There was no correlation between the OF rates and N concentrations of the shoots (*Figure 3A, Table 2*). This might be explained by the dilution of N taken up by the roots in plant tissues whose growth is enhanced with increasing rates of the OF (dilution effect; Jarrell and Beverly, 1981) because, in contrast, the concentration of OF application at increasing doses increased the uptake values per pot calculated with the formula concentration x dry weight in all placement methods (*Figure 4A*). A comparable situation was observed in a study conducted by Ayeni et al. (2012) on maize. In this study, the application of organomineral fertilizers, that is, the application of mineral nutrients together with the OF, increased the total N uptake but did not affect the N concentration.

In recent research, a study conducted by Shower (2019) with the broad bean plant in sandy soil revealed that the uptake of K by plants increased per unit area when applied with organic materials. This is also consistent with the present study results (*Figure 4C*). However, the lack of a clear relationship between the OF application and K concentration (*Figure 4C*) raises questions about how the OF increases K availability. The improvement in K uptake per pot may also occur due to the development of shoots, which can be explained by the dilution effect (Jarrell and Beverly, 1981) as described before.

Conclusions

In summary, the conclusions drawn from the results of the present study are presented as follows:

- The biogas facility-based OF positively affects wheat growth and nutrient uptake.
- Organic fertilizer application at 0.5-1.0 ton·ha⁻¹ with mineral nutrients increases the wheat's shoot growth by up to 15% when placed 5 cm below the surface (mimic of banding). The results of the correlation tests showed that there was a linear relationship between the shoot dry weight and OF rates.
- When the nutrient concentration in plant tissues, which is one of the indicators of plants' nutrient uptake, was evaluated, it was observed that only the P concentration could increase with the OF application.
- When mineral nutrients were applied 5 cm below the surface with the OF, the OF application increased the P concentration in plant tissues, unlike N and K. Like the shoot dry weight values, which were positively correlated with rising OF rates, P concentrations in the shoot tissues positively correlated with the OF rates. This situation can be explained in a way that P, which moves by diffusion in the soil, has limited mobility and binds quickly in the soil and may have increased its availability to the OF-applied plants. However, this issue needs to be scientifically demonstrated with additional laboratory, greenhouse, and field studies.

- When the effect of the OF on the nutrient contents in the shoot (uptake) is examined, it is seen that the contents of N and K, in addition to P, increase in parallel with the increasing plant growth, unlike the concentration. This indicates that the OF has a rising effect on the utilization efficiency of applied nutrients. And this effect is significant for the nutrients that cause environmental problems, such as N and P.

Acknowledgments. The present manuscript was prepared using the results of Kübra Dilan Hacırüstemoğlu's MSc thesis, which was granted by the Scientific Research Projects Coordination Unit at Çukurova University.

REFERENCES

- [1] Adewopo, J. B., VanZomerem, C., Bhomia, R. K., Almaraz, M., Bacon, A. R., Eggleston, E., Judy, J. D., Lewis, R. W., Lusk, M., Miller, B., Moorberg, C., Snyder, E. H., Tiedeman, M. (2014): Top-ranked priority research questions for soil science in the 21st century. – Soil Science Society of America Journal 78(2): 337-347. doi: 10.2136/sssaj2013.07.0291.
- [2] Amundson, R., Berhe, A. A., Hopmans, J. W., Olson, C., Sztein, A. E., Sparks, D. L. (2015): Soil and human security in the 21st century. – Science 348(6235): 647-653. doi: 10.1126/science.1261071.
- [3] Antille, D. L., Godwin, R. J., Sakrabani, R., Seneweera, S., Tyrrel, S. F., Johnston, A. E. (2017): Field-scale evaluation of biosolids-derived organomineral fertilizers applied to winter wheat in England. – Agronomy Journal 109(2): 654-674. doi: 10.2134/agronj2016.09.0495.
- [4] Atiyeh, R. M., Lee, S., Edwards, C. A., Arancon, N. Q., Metzger, J. D. (2002): The influence of humic acids derived from earthworm-processed organic wastes on plant growth. – Bioresource Technology 84: 7-14.
- [5] Ayeni, L. S., Edeleye, E. O., Adejumo, J. O. (2012): Comparative effect of organic, organomineral and mineral fertilizers on soil properties, nutrient uptake, growth and yield of maize (*Zea mays*). – International Research Journal of Agricultural Science and Soil Science 2: 493-497.
- [6] Ayinla, A., Alagbe, I. A., Olayinka, B. U., Lawal, A. R., Aboyeji, O. O., Etejere, E. O. (2018): Effects of organic, inorganic and organo-mineral fertilizer on the growth, yield, and nutrient composition of *Corchorus olitorius* (L.). – Ceylon Journal of Science 47(1): 13-19. doi: 10.4038/cjs.v47i1.7482.
- [7] Baldotto, L. E. B., Baldotto, M. A., Soares, R. R., Martinez, H. E. P., Venegas, V. H. A. (2012): Adventitious rooting in cuttings of croton and hibiscus in response to indolbutyric acid and humic acid. – Revista Ceres 59(4): 476-483. doi: 10.1590/S0034-737X2012000400007.
- [8] Baveye, P. C. (2015): Grand challenges in the research on soil processes. – Frontiers in Environmental Science 3: 1-5. doi: 10.3389/fenvs.2015.00010.
- [9] Brady, N. C., Weil, R. R. (2017): The Nature and Properties of Soils. – Pearson Education Limited, England.
- [10] Brannon, C. A., Sommers, L. E. (1985): Preparation and characterization of model humic polymers containing organic phosphorus. – Soil Biology and Biochemistry 17: 213-219. doi: 10.1016/0038-0717(85)90117-8.
- [11] Calvo, P., Nelson, L., Kloepper, J. W. (2014): Agricultural uses of plant biostimulants. – Plant and Soil 383: 3-41. doi: 10.1007/s11104-014-2131-8.
- [12] Canatoy, R. C., Daquiado, N. P. (2021): Fertilization influence on biomass yield and nutrient uptake of sweet corn in potentially hard setting soil under no tillage. – Bulletin of National Research Center 45: 61. doi: 10.1186/s42269-021-00526-w.

- [13] Cannales, L. P., Olivares, F. L. (2014): Physiological responses to humic substances as plant promoter. – *Chemical and Biological Technologies in Agriculture* 1: 3. doi: 10.1186/2196-5641-1-3.
- [14] Chen, Y., Aviad, T. (1990): Effects of humic substances on plant growth. – In: MacCarthy, P., Clapp, C. E., Malcolm, R. L., Bloom, P. R. (eds.) *Humic substances in soil and crop science: Selected readings*. American Society of Agronomy and Soil Science Society of America Madison, WI, pp. 161-186.
- [15] Cooper, R. J., Liu, C., Fisher, D. S. (1998): Influence of humic substances on rooting and nutrient content of creeping bentgrass. – *Crop Science* 38: 1639-1644. doi: 10.2135/cropsci1998.0011183X003800060037x.
- [16] Datta, R., Anand, S., Moulick, A., Baraniya, D., Pathan, S. I., Rejsek, K., Vranová, V., Sharma, M., Sharma, D., Kelkar, A., Formánek, P. (2017): How enzymes are adsorbed on soil solid phase and factors limiting its activity: A Review. – *International Agrophysics* 31(2): 287-302. doi: 10.1515/intag-2016-0049.
- [17] Delgado, A., Madrid, A., Kasem, S., Andreu, L., Del Campillo, M. D. C. (2002): Phosphorus fertilizer recovery from calcareous soils amended with humic and fulvic acids. – *Plant and Soil* 245: 277-286.
- [18] Ditta, A., Muhammad, J., Imtiaz, M., Mehmood, S., Qian, Z., Tu, S. (2018): Application of rock phosphate enriched composts increases nodulation, growth and yield of chickpea. – *International Journal of Recycling of Organic Waste in Agriculture* 7: 33-40. doi: 10.1007/s40093-017-0187-1.
- [19] Eden, M., Gerke, H. H., Houot, S. (2017): Organic waste recycling in agriculture and related effects on soil water retention and plant available water: a review. – *Agronomy for Sustainable Development* 37: 11. doi: 10.1007/s13593-017-0419-9.
- [20] Ehmann, A., Thumm, U., Lewandowski, I. (2018): Fertilizing potential of separated biogas digestates in annual and perennial biomass production systems. – *Frontiers in Sustainable Food Systems* 2:12. doi: 10.3389/fsufs.2018.00012.
- [21] Erdal, İ., Bozkurt, M. A., Çimrin, K. M., Karaca, S., Sağlam, M. (2000): Effects of humic acid and phosphorus applications on growth and phosphorus uptake of corn plant (*Zea mays* L.) grown in a calcareous soil. – *Turkish Journal of Agriculture and Forestry* 24: 663-668.
- [22] FAO (2009): How to feed the world in 2050. – Report on FAO Expert Meeting Rome 24-26 June 2009.
- [23] García, A. C., Berbara, R. L. L., Farias, L. P., Izquierdo, F. G., Hernández, O. L., Campos, R. H., Castro, R. N. (2012a): Humic acids of vermicompost as an ecological pathway to increase resistance of rice seedlings to water stress. – *African Journal of Biotechnology* 11(13): 3125-3134. doi: 10.5897/AJB11.1960.
- [24] García, A. C., Santos, L. A., Izquierdo, F. G., Sperandio, M. V. L., Castro, R. N., Berbara, R. L. L. (2012b): Vermicompost humic acids as an ecological pathway to protect rice plant against oxidative stress. – *Ecological Engineering* 47: 203-208. doi: 10.1016/j.ecoleng.2012.06.011.
- [25] Herencia, J. F., Maqueda, C. (2016): Effects of time and dose of organic fertilizers on soil fertility, nutrient content, and yield of vegetables. – *The Journal of Agricultural Science* 154(08): 1343-1361. doi: 10.1017/S0021859615001136.
- [26] Jarrell, W. M., Beverly, R. B. (1981): The dilution effect in plant nutrition studies. – In: Brady, N. C. (ed.) *Advances in Agronomy* 34: 197-224. doi: 10.1016/S0065-2113(08)60887-1.
- [27] Kominko, H., Gorazda, K., Wzorek, Z. (2017): The possibility of organo-mineral fertilizer production from sewage sludge. – *Waste Biomass Valorization* 8: 1781-1791. doi:10.1007/s12649-016-9805-9.
- [28] Lag, J., Hadas, A., Fairbridge, R. W., Muñoz, J. C. N., Pombal, X. P., Cortizas, A. M., Almendros, G. (2008): Humic Substances. – *The Encyclopedia of Earth Sciences Series* 315-323. doi: 10.1007/978-1-4020-3995-9_273.

- [29] Msangi, S., Rosegrant, M. (2009): World agriculture in a dynamically changing environment: IFPRI's long-term outlook for food and agriculture under additional demand and constraints. – FAO Expert Meeting on How to feed the World in 2050. Rome.
- [30] Mumbach, G. L., Gatiboni, L. C., de Bona, F. D., Schmitt, D. E., Dall'Orsoletta, D. J., Gabriel, C. A., Bonfada, E. B. (2019): Organic, mineral and organomineral fertilizer in the growth of wheat and chemical changes of the soil. – *Revista Brasileira de Ciências Agrárias* 14(1): e5618.
- [31] Nardi, S., Pizzeghello, D., Gessa, C., Ferrarese, L., Trainotti, L., Casadoro, G. (2000): A low molecular weight humic fraction on nitrate uptake and protein synthesis in maize seedlings. – *Soil Biology and Biochemistry* 32: 415-419.
doi: 10.1016/S0038-0717(99)00168-6.
- [32] Shafi, M. I., Adnan, M., Fahad, S., Wahid, F., Khan, A., Yue, Z., Danish, S., Zafar-ul-Hye, M., Brtnicky, M., Datta, R. (2020): Application of single superphosphate with humic acid improves the growth, yield and phosphorus uptake of wheat (*Triticum aestivum* L.) in calcareous soil. – *Agronomy* 10(9): 1224. doi: 10.3390/agronomy10091224.
- [33] Sharif, M., Burni, T., Wahid, F., Khan, F., Khan, S., Khan, A., Shah, A. (2013): Effect of rock phosphate composted with organic materials on yield and phosphorus uptake of wheat and mung bean crops. – *Pakistan Journal of Botany* 45(4): 1349-1356.
- [34] Shower, S. S. (2019): Effect of potassium fertilization and organic materials on some characteristic growth and nutrients uptake by faba bean (*Vicia Faba* L.) plant. – *Plant Archives* 19: 732-737.
- [35] Smith, W. B., Wilson, M., Pagliari, P. (2020): Organomineral fertilizers and their application to field crops. – In: Waldrip, M., Pagliari, P. H., He, Z. (eds.) *Animal Manure: Production, Characteristics, Environmental Concerns, and Management*. American Society of Agronomy and Soil Science Society of America Madison, pp. 229-243.
- [36] Steelink, C. (2002): Investigating humic acids in soils. – *Analytical Chem.* 74: 326A-333A.
doi: 10.1021/ac022040m.
- [37] Steiner, C., Teixeira, W. G., Lehmann, J., Nehls, T., de Macêdo, J. L. V., Blum, W. E. H., Zech, W. (2007): Long term effects of manure, charcoal and mineral fertilization on crop production and fertility on a highly weathered Central Amazonian upland soil. – *Plant and Soil* 291(1-2): 275-290. doi:10.1007/s11104-007-9193-9.
- [38] Tahir, M. M., Khurshid, M., Khan, M. Z., Abbasi, M. K., Kazmi, M. H. (2011): Lignite-Derived humic acid effect on growth of wheat plants in different soils. – *Pedosphere* 21: 124-131. doi: 10.1016/S1002-0160(10)60087-2.
- [39] Visser, S. (1986): Effects of humic substances on plant growth. – In: Burns, R., Dell'Agnola, G., Miele, S., Nardi, S., Savoini, G., Schnitzer, M., Sequi, P., Vaughan, D., Visser, S. (eds.) *Humic substances, effect on soil and plant*. Reda Edizioni per L'agricoltura, Milan, Italy, pp. 89-107.
- [40] Zandonadi, D. B., Busato, J. G. (2012): Vermicompost humic substances: technology for converting pollution into plant growth regulators. – *International Journal of Environmental Science and Engineering Research* 3: 73-84.
- [41] Zandonadi, D. B., Santos, M. P., Busato, J. G., Peres, L. E. P., Façanha, A. R. (2013): Plant physiology as affected by humified organic matter. – *Theoretical and Experimental Plant Physiology* 25: 12-25.