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EFFECT OF HUMIC+FULVIC ACID APPLICATION AT DIFFERENT DOSES ON BIOLOGICAL ACTIVITY OF DIFFERENT REGION SOILS

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Abstract

An organic matter content of soil is one of the major parameter representing soil quality. A pot experiment carried out to determine the effect of humic+fulvic acid (HFA) is considered to be the most effective part of the organic matter on biological activity of different region soils. In this study, HFA doses of 0, 500, 1000, 2000 ppm were applied to soil collected from 7 different regions of Turkey and corn seeds were sown. Following the harvest, the colony forming units of microorganisms, CO_2 production, dehydrogenase enzyme activity (DHA) and microbial biomass carbon (MBC) were analysed to evaluate biological activity of soils. The results obtained revealed that the HFA was effective in terms of parameters determined but, the effectiveness was variable in terms of the region studied. The lower values were observed in the soil taken from Konya region according to the biological activity parameters of CO_2 production, DHA and MBC. The higher values were determined in soil taken from Antalya or Samsun. According to HFA doses, increment until 1000 ppm resulted in significantly higher values of biological activity parameters whereas, increment from 1000 to 2000 ppm was not effective on the parameters mentioned above, even decline was observed in some cases.

Key words: *maize, biological activity, humic and fulvic acid.*

INTRODUCTION

Organic matter improves soil physical, chemical and biological properties of the soil (Shirani et al., 2002); however, soil organic matter contents of Turkey generally is low (Eyupoglu, 1998; Gezgin et al., 1999). Thus, any practice targeted to increase organic matter contents of soil has great importance in Turkey to ameliorate aggregate stability, water-air balance, resistance to erosion as well as plant nutrition. Influence of humic substance on plant nutrition occurs indirectly via increasing water holding capacity, aeration and availability of nutrients or directly via stimulating root development, chelating the metals and changing the uptake metabolism (Lobartini et al., 1997). Humic and fulvic acids (HFA) also have a challenge on soil reclamation, bioremediation and odor prevention (Gunes, 2007). Seker and Ersoy (2005) pointed that dual applications of mineral fertilizer and humic substance increased N, P, K, Fe, Zn, and Mn contents of the *Zea mays*. Effects of humic substances are related to many factors. Leventoglu and Erdal (2014) reported close relation between soil type and humic substance applications on dry weight, N, Mg, and Cu concentrations of the corn. Sozudogru et al. (1996) reported positive effects of humic acids on N and P uptake whereas no effects on K, Ca, Na and Cu. Furthermore, Erdal et al. (2014) reported negative effects on Fe uptake which is one of the problematic elements on plant uptake in Turkey. Soil biologic characteristic is also affected by humic substance applications. Visser (1985) reported 200 times more microorganism number in case of 30 mg L^{-1} HFA applications. Turgay et al. (2004) reported higher CO_2 formation in leonardite application which has a considerable amount of humic substances. The aim of this research was to evaluate humic substances on soil biologic activity with respect to different region soils.

MATERIALS AND METHODS

This research was carried out as a pot experiment at Suleyman Demirel University, Soil Sci. & Plant Nutrition Dept. Soils are collected from 7 different regions of Turkey, whose brief properties are provided in Table 1. Dracme corn variety was used as a test plant.
Humic+fulvic acid extracted from leonardite

using KOH. Doses were 0, 500, 1000, and 2000 mg kg^{-1} soil and they were mixed thoroughly afterwards placed to pots containing 1500 g soil. Basic fertilization was performed as 300 mg kg^{-1} N, 200 mg kg^{-1} P, and 200 mg kg^{-1} K using NH_4NO_3, TSP, K_2SO_4 and diammonium phosphate. At the end of the experiment, soils were analyzed to determine their CO_2 production (Isermayer, 1952), microbial biomass carbon contents (Ohlinger, 1993), dehydrogenase activity (Beyer et al., 1993) and number of microorganism (Ottow, 1984). All results statistically were analyzed via MSTAT-C pocket software.

Table 1. Some properties of the soil used

Properties of soil	Diyarbakir	Konya	Urfa	Samsun	Kutahya	Eskisehir	Antalya
Sand (%)	39.8	37.7	28.0	57.5	65.2	41.5	21.7
Silt (%)	22.0	25.5	20.0	14.1	21.4	12.2	25.9
Clay (%)	38.2	36.8	52.0	28.4	13.4	46.3	52.4
Tex.class	C	CL	SCL	SCL	C	SC	SCL
Corg (%)	1.64	0.54	1.56	1.27	1.29	0.88	2.46
$CaCO_3$(%)	34.4	22.8	41.3	8.3	28.5	27.4	22.3
pH	8.3	8.3	8.5	7.4	8.1	8.1	7.9
Salt (%)	0.36	0.16	0.61	0.09	0.30	0.31	0.10

RESULTS AND DISCUSSIONS

The effects of humic+fulvic acid (HFA) applications to different regions of soils on CO_2 production presented in Table 2.

According to dose averages, all applications increased CO_2 production of the soils, where the statistically the lowest value was observed in the control. No statistical differences were determined between doses. Averages of regions represent considerable differences; Diyarbakir gave nearly two times higher value than that of Konya soils. The highest and the lowest values in dose x region interaction were in Samsun soil with 2000 mg kg^{-1} HFA application and control variant of Konya soil, respectively. Dehydrogenase activities (DHA) of soils are given in Table 3.

Comparing to CO_2 production, DHA was not as effective at the lower doses of HFA. The highest value was observed in middle dose, the highest dose even diminished for the DHA comparing to control. Region-average data revealed that the highest DHA was in Antalya and the lowest were in Konya and Kutahya soils. The highest dose x region interaction was also in Antalya and more than 30 times higher than the lowest value which was observed in control variant of Konya. Control soil of Konya yielded the lowest value in both CO_2 production and DHA; however the highest values were determined from different regions. Similarly CO_2 production and DHA, Microbial Biomass Carbon (MBC) were greatly influenced from HFA applications (Table 4).

Table 2. CO_2 production (µg CO_2-C g_dry_soil^{-1} 24 h^{-1})

Regions	Doses: 0 (control)	500 mg kg^{-1}	1000 mg kg^{-1}	2000 mg kg^{-1}	Average
Diyarbakir	26.1 e-i	34.2 a-c	37.2 a	33.1 a-e	32.6 A
Konya	8.8 m	16.8 j-l	25.5 f-i	14.7 k-m	16.5 D
Urfa	25.5 f-i	26.6 d-i	32.6 a-f	13.2 lm	24.5 C
Samsun	22.8 h-j	28.4 c-h	22.1 h-j	37.4 a	27.7 B
Kutahya	22.1 h-j	31.7 a-f	31.0 a-g	35.8 ab	30.1 AB
Eskisehir	22.2 h-j	24.3 g-i	20.6 i-k	29.0 b-h	24.0 C
Antalya	28.4 b-h	33.6 a-d	29.2 b-h	29.3 b-h	30.1 AB
Average	22.3 B	27.9 A	28.3 A	27.5 A	

Table 3. Dehydrogenase activity (µg TPF 10 g_dry_soil^{-1} 24 h^{-1})

Regions	Doses: 0 (control)	500 mg kg^{-1}	1000 mg kg^{-1}	2000 mg kg^{-1}	Average
Diyarbakir	163.1 f-k	178.7 f-j	136.6 g-m	208.3 fg	171.7 D
Konya	35.0 m	93.8 i-m	70.1 k-m	57.2 l-m	64.0 E
Urfa	184.1 f-i	197.4 f-h	212.9 fg	141.7 g-l	184.0 D
Samsun	456.3 d	458.2 d	354.7 e	372.6 de	410.4 B
Kutahya	96.9 h-m	80.2 g-m	77.1 j-m	87.2 i-m	85.4 E
Eskisehir	204.4 fg	257.3 f	239.8 fg	223.5 fg	231.2 C
Antalya	1013.3 b	914.1 c	1182.7 a	846.3 c	989.1 A
Average	307.6 AB	311.4 AB	324.8 A	276.7 B	

Table 4. Microbial Biomass Carbon-MBC (μg MBC g_dry_soil^{-1})

Regions	Doses 0 (control)	500 mg kg^{-1}	1000 mg kg^{-1}	2000 mg kg^{-1}	Average
Diyarbakir	198.3 ab	122.6 b-e	176.7 a-c	108.2 c-f	151,4 B
Konya	25.2 fg	86.5 d-g	97.3 c-g	137.0 b-d	86,5 CD
Urfa	43.3 e-g	54.1 d-g	108.2 c-f	68.5 d-g	68,5 D
Samsun	230.8 a	238.0 a	248.8 a	245.2 ab	240,7 A
Kutahya	82.9 d-g	39.7 e-g	21.6 g	39.7 e-g	46,0 D
Eskisehir	104.6 c-g	22.4 g	115.4 c-e	113.7 c-e	89,0 CD
Antalya	93.7 c-g	111.8 c-e	194.7 ab	122.6 b-e	130,7 BC
Average	111.3 AB	96.4 B	137.5 A	119.3 AB	

MBC as a parameter of microbial organism presence of soil revealed that the middle dose was more effective than higher and lower doses. According to dose-average values, lower doses even reduced MBC comparing to control. Samsun showed the highest region-average value whereas the highest region x doses interaction value also was determined in that region. Similar to CO_2 production and DHA, the lowest MBC value was determined in Konya once again. This data had a great importance to represent natural situation of Konya soils, which had quite low biological activity. For all region sampled, soil bacteria, fungi and actinomycetes numbers were determined and the results are presented in Figure 1, 2, 3 respectively.

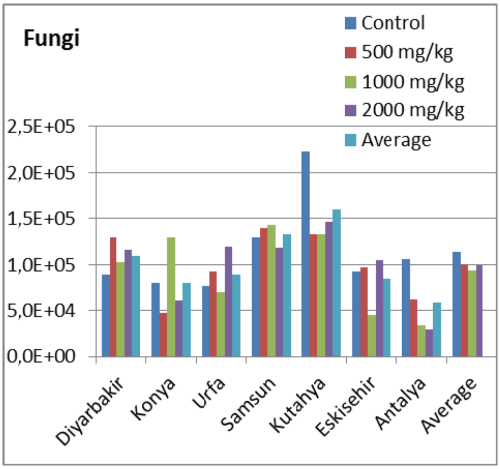

Figure 2. The number of fungi in one g of soil

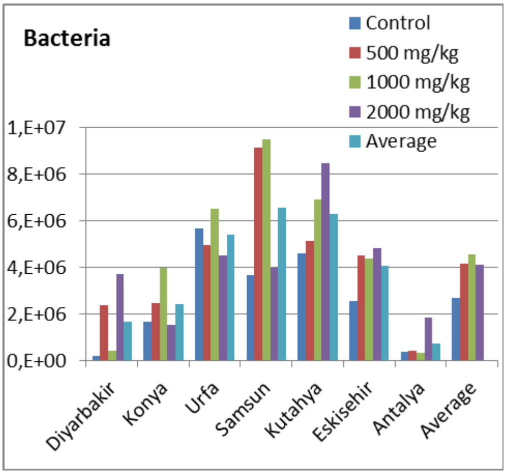

Figure 1. The number of bacteria in one g of soil

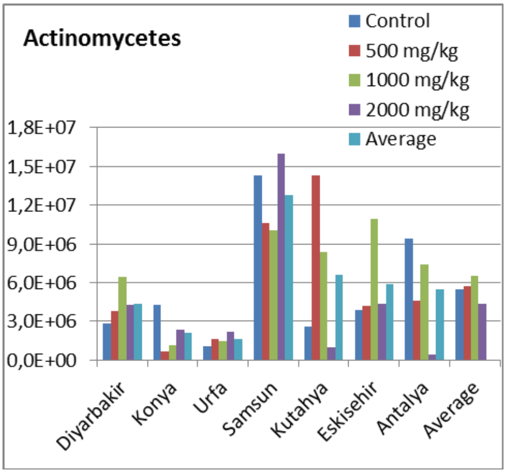

Figure 3. The number of actinomycetes in one g of soil

As clearly seen in Figure 1, higher colony forming unit (cfu) of bacteria was observed in Samsun soil. Antalya gave the lowest values in general. Dose-average values revealed that all doses increased bacteria abundance; however, 1000 mg kg^{-1} dose was more effective among the doses. Fungi cfu (Figure 2) was not in accordance with bacteria based on dose-averages. All doses reduced fungi abundance. The highest fungi was determined in Samsun and Kutahya whereas the lowest was found in Antalya. Diminishing effect of HFA application was more prominent in Antalya soils. Similar to fungi results, cfu of actinomycetes was the highest in Samsun and Kutahya whereas the lowest were in Konya and Urfa. In general quite much fluctuation was determined in soil microorganism's abundance. To compare the natural situation of the regions, data observed in control is represented in one table (Table 5).

Table 5. Natural situation of the regions in term of biological parameters

Regions	CO_2 (µg CO_2-C gds^{-1} 24 h^{-1})	DHA (µg TPF 10gds $24h^{-1}$)	MBC (µg MBC gds^{-1})	Fungi cfu x 10^4/gds	Bacteria cfu x 10^5/gds	Actinomycete cfu x 10^6/gds
Diyarbakır	26.1	163.1	198.3	9.0	1.7	2.8
Konya	8.8	35.0	25.2	8.0	16.6	4.3
Urfa	25.5	184.1	43.3	7.7	56.7	1.1
Samsun	22.8	456.3	230.8	13.0	36.9	14.3
Kütahya	22.1	96.9	82.9	22.3	46.0	2.6
Eskişehir	22.2	204.4	104.6	9.3	25.4	3.9
Antalya	28.4	1013.3	93.7	10.7	3.6	9.4

(gds: gram dry soil; cfu: colony forming unit)

The lowest CO_2 production, DHA and MBC values, which are the predominant parameters of biological activity, were determined in Konya soil. Konya is located nearly in the middle of Turkey with less precipitation accompanying relatively colder climate region. This would be the reason of less biologic activity in Konya. Supporting this idea, Antalya, which has higher precipitation and warmer climate, gave higher CO_2 and DHA. Among the regions, the highest precipitation occurred in Samsun, which the highest MBC recorded. Table 5 clearly indicated the great biological differences in the region; therefore, the effect of HFA varied in quite wide range.

The correlations between the parameters are presented in Table 6.

Table 6. Correlations between parameters determined

	CO_2	DHA	MBC	Fungi	Bacteria
DHA	0.2401 *				
MBC	0.1274	0.3068 **			
Fungi	0.1154	-0.2794 *	0.0554		
Bacteria	0.0669	-0.2821 *	-0.0222	0.3626 ***	
Actinomycete	0.1736	0.1881	0.3426 **	0.1859	0.0889

*: $p<0.05$ **: $p<0.01$ ***$p<0.001$

As seen from Table 6, there was a positive correlation between DHA and CO_2 production at $p<0.05$ level. No more significant correlation with CO_2 production was observed. DHA is an enzyme that has a role on respiration; therefore, correlation between them is not extraordinary, even higher correlation coefficient was expected. There was a significant positive correlation ($p<0.01$) between soil MBC indicating the total amount of microorganisms and DHA activity whereas DHA gave negative correlation with fungi and bacteria. The strongest correlation was determined between fungi and bacteria at $p<0.001$ level. Actinomycete was only correlated by MBC with $p<0.01$. Actinomycetes are more resistant to heat and drought stress, thus in all regions actinomycete abundance was relatively high. In normal cases most available group of microorganisms consisted by bacteria, this is the evidence of the stress condition of the some areas studied.

CONCLUSIONS

Humic+fulvic acid is a part of organic matter which recently became very popular in Turkey. There are a number of associations and research centres all over the world to evaluate complex structure of the humic substances. Although HFA is a part of organic matter, considering recommended dose, it does not have potential to improve soil organic matter contents. However, when applied in even small quantities, it is still effective due to its hormone-like effect. On the other hand, optimum dose for every type of soil is yet not known. Therefore this research was carried out to evaluate relation between application doses and different soils from different regions.

Results revealed that different soils react considerably different up to HFA doses. As an example, effect of HFA application on CO_2 production was much more effective in Konya soil comparing to all others. Even the lowest dose of HFA increased CO_2 formation as double in this region. Determined CO_2 production of untreated Konya soil provided only 1/3 of the other region indicating insufficient biological productivity of soils which means HFA has a great importance in that areas.

In general, HFA is defined as a potential agent to improve soil biologic parameters based of the data obtained in this research. Optimum doses were 500 mg kg^{-1} for Antalya, 2000 mg kg^{-1} for Samsun, Kutahya and Eskisehir, and 1000 mg kg^{-1} for rest of the region studied. Although higher doses above 2000 mg kg^{-1} were not tested, changes on CO_2 production by increasing doses indicated that the higher doses had a potential to decrease marginal CO_2, thereby biological activity. DHA values also gave similar results to the CO_2; however, the highest value on CO_2 production was in Samsun while the highest DHA was in Antalya. As mentioned earlier, DHA had a role on respiration, thus close relations are expected between CO_2 production and DHA which it was week in this research. However, Beck (1984) reported extraordinary circumstances controlled by many factors that preventing close relation between CO_2 and DHA. In accordance with CO_2 and DHA, the lowest MBC value is also determined in Konya soil. This situation is most probably related by low organic matter contents as well as cold climate and drought condition in that region. Proving this idea, the highest MBC determined in Samsun soil having uniform precipitation throughout the year provided water requirement of soil microorganisms and led to organic matter accumulation. CO_2 production data was also confirmed with MBC values for Samsun. Close relation between MBC and soil organic carbon contents reported many times in the literature (Schnurer el al., 1985; Sparling et al., 1986; Franzluebbers et al., 1995; Okur et al., 2006). Moreover Hasebe et al. (1985) reported the highest MBC in organically fertilized soils. Organic fertilizer and green manure applications increased soil C, N and MBC contents as well as CO_2 production DHA and mineralization (Frazer et al., 1988; Bhardjaw and Datt, 1995). Results obtained from this research also represented that the HFA application as a part of organic matter increase CO_2, DHA and MBC.

Based on the results gathered, no doubt, HFA applications increased soil biological activity; however excessive doses diminishing soil biological parameters. Therefore, regional dose determination researches should be carried out to prevent unwanted circumstances caused by high rate HFA applications.

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