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Microbiological Indicators of a Clayey Soil Planted with Wheat (*Triticum aestivum* L.) as Affected by Potassium Fertilization and Different Water Regimes

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ABSTRACT

A greenhouse trial was carried out in Department of Soil and Water Sciences, Faculty of Agriculture, Benha University, Egypt from November 2012 to May 2013 to determine the effect of different potassium rates and water regimes on soil microbial properties in a clayey soil. The experiment was factorial with twelve treatments (4 potassium rates×3 water regimes) in triplicates. Three soil microbial indicators were evaluated, microbial biomass (carbon and nitrogen), microbial population (bacteria and fungi) and enzyme activities (urease, phosphatase, catalase and dehyrdogenase). Moreover, the cumulative CO_2 was chosen as a microbial indicator and estimated during an incubation experiment for 120 days under natural temperature. Soil microbial properties were determined four times during the experimental work. Significant increases in soil microbial biomass, microbial population and enzyme activities were recorded after addition of potassium fertilizer and these improvements became generally more noticeable with the increase of potassium applied rates. Changes of soil moisture contents caused marked effects on soil microbial biomass, microbial population and enzyme activities. Cumulative CO_2 was also affected by potassium and soil water rates and its highest values were found after 120 days of incubation. Both soil water levels and potassium fertilization rates should be considered as good factors in governing soil biological properties in clayey soils.

Key words: Potassium, soil moisture, microbial properties, fertilization, clayey soil

INTRODUCTION

Soil microorganisms such as bacteria and fungi have indispensable roles in the biosphere and soil environmental system because they are decisive factors in controlling soil biochemical processes, including organic matter decomposition, nutrient cycling and bioremediation of soil pollution (Larkin, 2003; Tejada *et al*., 2007). Alterations in the population and diversity of soil microorganisms are responsive and strong indicators of soil biological activity, soil health and quality, plant productivity and ecosystem sustainability (Doran and Parkin, 1994; Edmeades, 2003). Evaluation of soil microbial properties, including biomass, activity and community is considered as an important task for improving our knowledge about the governing factors for soil health and quality (Garcia *et al*., 1997; Hill *et al*., 2000; Saviozzi *et al*., 2002;

Ros *et al*., 2003). Soil enzymes have vital roles in nutrient cycling processes and decomposition of organic matter (Dick *et al*., 1994; Dick, 1997) and the enhancement of their activities can be used as a good indicator for soil fertility and quality (Ndiaye *et al*., 2000).

Mineral fertilizers are important amendments and nutrient sources for plant growth in the agricultural system. Research in this area is generally focused on the improvements of crop yields but the cumulative effects of mineral fertilizers on soil biology are usually neglected. The influence of fertilizers on soil microbial properties depends on many factors such as counts and types of microorganisms, soil type, soil physical and chemical properties, rates and forms of fertilizers and application time (Cerny *et al*., 2003; Mandal *et al*., 2007; Stark *et al*., 2007; Ge *et al*., 2008). There are some studies have been conducted to evaluate the effect of fertilization on soil microbial properties and found that application of mineral fertilizers can increase soil enzyme activities (Li *et al*., 2011; Simon and Czako, 2014). However, other studies showed that mineral fertilizers can decrease soil quality (Doran *et al*., 1996; Zhang *et al*., 2015) or have no effect on soil microbial properties (Aref and Wander, 1997; Treseder, 2008). There is also growing evidence that soil biological properties are influenced by environmental factors and can be potential indicators of ecological stress (Dick and Tabatabai, 1992).

Soil moisture has a vital role in controlling soil biota and the change of its levels can affect microbial properties such as enzyme activities, microbial population and microbial biomass (Ma *et al*., 2012; Hassan *et al*., 2013). The deficiency of soil water content has adverse effects on soil microorganisms through microbial death and cell lysis (Hassan *et al*., 2013). Microbial properties are highly and generally tied to soil conditions such as soil texture, water retention, porosity, soil depth, water content, temperature and pH (Goncalves and Carlyle, 1994; Rodrigo *et al*., 1997; Leiros *et al*., 1999; Sinegani and Mahohi, 2010). In semiarid environment, soil microbial activity is particularly affected by the water availability, since the good presence of water has positive influence on respiration and nutrient mineralization processes (Collins *et al*., 2008). Ma *et al*. (2012) reported that alterations of soil water content can cause significant changes in physiology and growth of soil microbial communities by affecting nutrients availability and oxygen concentrations. To our knowledge, managements of water content and K fertilization in clayey soils are important factors for improving soil quality indicators. Therefore, this experimental work aims to assess the potential impact of soil water content and different K levels on microbial properties in a clayey soil planted with common wheat (*Triticum aestivum*).

MATERIALS AND METHODS

Soil: Surface soil samples (0-30 cm) were collected in October 2012 from the Experimental Farm of Faculty of Agriculture, Benha University, Kalubia Governorate, Egypt. Soil samples were passed through a 2 mm sieve and divided into two parts. The first part was air dried for one week at room temperature for determination of soil physical and chemical properties, while the second part was stored at 4°C for microbial analyses. The physical, chemical and microbial properties of the studied clayey soil are given in Table 1.

Experimental work: A pot experiment was conducted from November 2012 to May 2013 in the greenhouse of Department of Soil and Water Sciences, Faculty of Agriculture, Benha University, Egypt. The experiment was factorial with two factors and contained 12 treatments resulted from the combination of four potassium rates (0, 25, 50 and 75 mg kg^{-1}) and three water regimes: W1 = 40%, W2 = 55% and W3 = 70% of Water-Holding Capacity (WHC). The experimental pots

Table 1: Some properties of the studied clayey soil

Properties	Values
Physical	
Sand (%)	14.84
Slit (%)	25.74
Clay (%)	59.42
Texture	Clay
SOM (g kg^{-1})	25.7
$CaCO_3$ (g kg^{-1})	15.26
B.D (g cm^{-3})	1.38
Chemical	
pH	7.96
EC (dS m^{-1})	1.85
Available N (mg kg^{-1})	49.60
Available P (mg kg^{-1})	28.30
Available K (mg kg^{-1})	128.00
Total N (g kg^{-1})	1.03
Total P (g kg^{-1})	0.39
Total K (g kg^{-1})	1.48
Biological	
Urease	1.97
Catalase	0.08
Alk. Phos.	189.00
Dehydrogenase	11.70
MBC (mg kg^{-1})	208.00
MBN (mg kg^{-1})	46.30
Total bacteria (10^{10} CFU g^{-1})	2.64
Total fungi (10^3 CFU g^{-1})	5.11

EC: Electrical conductivity, SOM: Soil organic matter, Alk. Phos.: Alkaline phosphatase, B.D: Bulk density, measured units of urease, catalase, alkaline phosphatase and dehydrogenase are mg NH_4 kg^{-1} h^{-1}, 0.01 M $KMnO_4$ mol L^{-1}, mg pNP kg^{-1} h^{-1}, mg TPF kg^{-1} h^{-1}, respectively. MBC: Microbial biomass carbon, MBN: Microbial biomass nitrogen

(20×20 cm) were filled with 4 kg of the above mentioned clayey soil and then organized in a complete randomize block design. Wheat (*Triticum aestivum* L.) var. Sakha 93 was used as an indicator plant in this experiment. The grains were obtained from Department of Agronomy, Faculty of Agriculture, Benha University, Egypt. Ten grains of wheat were sown in each pot and then the pots were irrigated with tap water. After 15 days, seedlings of wheat were thinned and 5 plants were kept in each pot.

Soil sampling: Soil samples were taken on intervals (30, 60 and 120 days after wheat thinning and after the harvest of wheat) using an auger at five randomly selected spots, mixed well in plastic bags and transferred directly to the laboratory. After that, soil samples were passed through a 2 mm sieve and then stored at 4°C for microbial analyses.

Basic soil physical and chemical analyses: The particle-size distribution of soil was measured by the pipette method (Kettler *et al*., 2001). The pH was analyzed in a suspension of 1:2.5 soil/water (w/v), while soil Electrical Conductivity (EC) was determined in the extraction solution of 1:1 soil/water (w/v). Total Organic Carbon (TOC) was estimated in the presence of $K_2Cr_2O_7$ and concentrated H_2SO_4 followed by ferrous ammonium sulfate $(NH_4)_2$ Fe $(SO_4)_2.6H_2O$ titration (Yeomans and Bremner, 1998). A factor of 1.72 was used to convert TOC to SOM. Total N, P and K were determined after samples digestion by a concentrated mixture of H_2SO_4-$HClO_4$ (2:1, v/v). Available N, P and K were extracted by KCl (2 mol L^{-1}), $NaHCO_3$ (0.5 mol L^{-1}, pH = 8.5) and NH_4OAc (1 mol L^{-1}, pH = 7.0). Total and available N values were

determined by the Kjeldahl method (Hinds and Lowe, 1980), while amounts of total and available P were measured using the spectrophotometer in blue molybdate-phosphate complexes in the presence of ascorbic acid (Murphy and Riely, 1962). The flame-photometer was used to detect total and available K. Total $CaCO_3$ was measured by estimating quantity of CO_2 that produced after addition of HCl to the soil (Dewis and Freitas, 1970). Soil bulk density was determined by the core method (Blake, 1965). The soil was dried at 105°C for 48 h and the bulk density was calculated as the ratio between soil dry weight and the cylinder volume.

Cumulative soil CO_2: An incubation experiment was conducted in plastic jars (1 L) for 120 days under natural temperature in the dark to estimate the cumulative soil CO_2. The jars were covered with plastic films that contained small holes for air exchange between the soil and the atmosphere. Carbon dioxide that produced from the respiration process was trapped in NaOH (2 mol L^{-1}). The residual NaOH was titrated with HCl in the presence of phenolphthalein as an indicator (Anderson, 1982). The experiment contained control jars without soil. Values of cumulative CO_2 were determined after: 5, 10, 20, 40, 80 and 120 days.

Enzyme activities: Fresh soil samples were collected to determine four soil enzyme activities. Dehydrogenase (DHA) was determined as described in Friedel *et al*. (1994) after soil incubation at 37°C for 24 h with 2,3,5-triphenyl-tetrazolium chloride (TTC) and the red color of triphenylformazan (TPF) absorbance was measured at 546 nm using spectrophotometer. Catalase (CAT) activity was measured by titration of the excess of H_2O_2 by $KMnO_4$ (0.1 mol L^{-1}) in the presence of H_2SO_4 following method of Johnson and Temple (1964). Urease activity was determined in the form of NH_4^+ during incubation of soil samples for 3 h with urea (0.2 mol L^{-1}) as a substrate at 37°C in Tris buffer (pH = 9) and the absorbance of blue color was checked at 578 nm by the spectrophotometer (Tabatabai and Bremner, 1972). Alkaline phosphatase activities were measured after the soil incubation for 1 h at 37°C with a 0.025 mol L^{-1} p-nitrophenyl phosphate (pNPP) substrate and Modified Universal Buffer (MUB) (0.17 mol L^{-1}), at pH 11 and finally yellow color of the released p-nitrophenol (pNP) was estimated at wavelength of 400 nm with a spectrophotometer (Tabatabai and Bremner, 1969).

Microbial biomass carbon and nitrogen: The collected fresh soil samples were separated into two parts. The first part (non-fumigated) was directly extracted by K_2SO_4 (0.5 mol L^{-1}). The second part (fumigated) was put in Petri dishes and transferred to a desiccator had 30 mL $CHCl_3$ for fumigation. A vacuum pump was used to increase the pressure until the beginning of $CHCl_3$ boiling. The desiccator was kept for 24 h in darkness at 25°C and then soil samples extracted also by K_2SO_4 (0.5 mol L^{-1}) (Brookes *et al*., 1985). The concentrations of C and N in non-fumigated and fumigated samples were determined by $K_2Cr_2O_7$ oxidation (Yeomans and Bremner, 1998) and Kjeldahl method (Hinds and Lowe, 1980), respectively. Values of microbial biomass C and N were calculated as the difference between the fumigated and non-fumigated extracts and then divided by 0.45 (K_C) (Beck *et al*., 1997) and 0.54 (K_N) (Brookes *et al*., 1985).

Total microbial count: Total bacteria and fungi were estimated using dilution plate method as described by Hu *et al*. (2013). A soil sample of 10 g was shaken with 100 mL sterile water for 20 min at 250 rpm. For bacteria, the medium of Luria Broth (LB) that contained 5 g NaCl, 5 g tryptone, 2.5 g yeast extract and 7.5 g agar was used. The fungi was cultured on Martin's rose Bengal

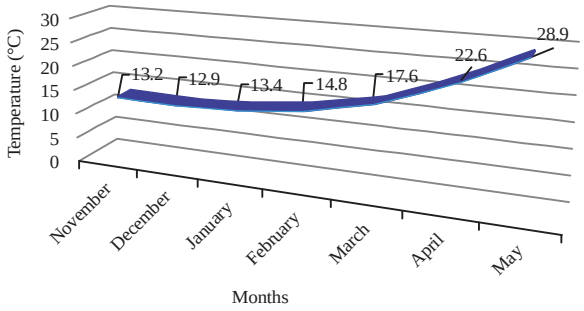

Fig. 1: Mean of temperature during the experimental season of wheat from November 2012 to May 2013

streptomycin medium that contained 10.0 g glucose, 5.0 g peptone, 1.0 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 33 mg rose Bengal and 3.0 mL 1% streptomycin. The experimental plates were incubated at 28°C for 2 days for the assay of bacteria and 4 days for fungi. The total counts of the bacteria and fungi were expressed as CFU g^{-1} dry soil.

Temperature changes: Monthly changes of temperature were measured during the wheat growing season from November 2012 to May 2013 and recorded in Fig. 1.

Statistical analysis: Data was statistically analyzed by Statistica 8.1 for Windows software. A two-way analysis of variance was performed to examine the main effect of K fertilizer rates and water regimes on soil properties. The significant differences between the treatments were evaluated using Least Significant Difference (LSD) test at $p<0.05$.

RESULTS AND DISCUSSION

Cumulative CO_2: Data in Table 2 showed the effect of water regimes and K rates on cumulative CO_2 during an incubation experiment for 120 days. The increase of K rates from 0-75 mg kg^{-1} was responsible for considerable improvements in cumulative CO_2. The effect of K2 (medium dose) on cumulative CO_2 was higher than those of K1 and K3. This could be used to confirm the significant role of K2 in soil respiration that expressed by cumulative CO_2 as compared with K1 and K3 additions. Changes of the water regime at K1, K2 and K3 were also significantly affected cumulative CO_2. However, when the soil did not treat with K (K0), increase of soil water levels from 40-55% of WHC (W1 and W2) had no significant effect on cumulative CO_2 at all incubation periods. Generally, the use of 70% of WHC (W3) at all K rates caused higher enhancements in cumulative CO_2 compared to W1 and W2. These results are in harmony with the findings of Iqbal *et al*. (2009), who showed that application of water at 60 and 80% of WHC caused higher increases in soil respiration in comparison to 20 and 40% of WHC in paddy, upland, woodland and orchard soils. Hassan *et al*. (2014) found that alteration of soil moisture is considered as a vital factor in controlling soil respiration and then cumulative CO_2 in Ultisol soil. The highest values of cumulative CO_2 were noticed at K2W3 treatment (268, 478, 685, 894, 1042 and 1279 mg kg^{-1} soil), whereas the lowest values were found at K0W1 (128, 155, 204, 276, 331 and 380 mg kg^{-1} soil) after 5, 10, 20, 40, 80 and 120 days, respectively. Increasing incubation times showed high effects on cumulative CO_2 and its highest values were recorded after 120 days at all rates of K and water regime. This could be explained by the release of carbon during the decomposition process of soil

organic matter. Also, addition of K fertilizer at different rates under good water regime might improve values of cumulative CO_2 due to the increase of organic matter mineralization and nutrients releasing. Tejada *et al*. (2007) reported that soil respiration could be one of the best indicators of soil microbial properties and provided important information about amounts of easily decomposable substrates. The highest values of cumulative CO_2 after 120 days could be also due to the increase of temperature in these periods. Increase of temperature could strongly enhance soil respiration that illustrated by cumulative CO_2 as a result of high microbial activity and mineralization of soil organic matter (Iqbal *et al*., 2009; Hassan *et al*., 2014).

Microbial properties: All enzyme activities (urease, catalase, alkaline phosphatase and dehydrogenase), microbial biomass carbon and nitrogen (MBC and MBN) and microbial counts (bacteria and fungi) were strongly affected by changes of K fertilization and water rates (Table 3-6). These soil properties were commonly used as good and insightful indicators to environmental stress, including drought and nutrients deficiency (Dick, 1997). In the soil environment, most of reactions were catalyzed by enzymes. Soil enzymes could be considered as potential indicators of soil quality and strongly influenced microbial activity and nutrient cycling (Dick *et al*., 1996). Dehydrogenase was one of the intracellular enzymes that used as a valid indicator of soil

Table 2: Cumulative CO_2 (mg kg^{-1} soil) during incubation of a clayey soil treated with different rate of potassium fertilizer and water regimes

	Incubation periods (days)					
Treatments	5	10	20	40	80	120
K0W1	128.00	155.0	204.0	276.0	331.0	380.0
K0W2	135.00	167.0	212.0	290.0	348.0	403.0
K0W3	147.00	184.0	235.0	314.0	375.0	436.0
K1W1	159.00	241.0	368.0	512.0	604.0	667.0
K1W2	184.00	273.0	415.0	578.0	680.0	718.0
K1W3	212.00	316.0	460.0	641.0	762.0	820.0
K2W1	191.00	362.0	543.0	726.0	840.0	1016.0
K2W2	226.00	411.0	609.0	802.0	936.0	1125.0
K2W3	268.00	478.0	685.0	894.0	1042.0	1279.0
K3W1	180.00	266.0	401.0	559.0	647.0	786.0
K3W2	201.00	303.0	452.0	617.0	821.0	995.0
K3W3	222.00	350.0	510.0	684.0	1006.0	1224.0
LSD (0.05)	9.14	12.8	20.9	26.3	35.6	43.7

K0, K1, K2 and K3 = 0, 25, 50 and 75 mg kg^{-1}, W1, W2 and W3 = 40, 55 and 70% of water-holding capacity, respectively, LSD: Least significant difference

Table 3: Dehydrogenase and catalase activities in wheat rhizosphere under different rates of potassium fertilizer and water regimes

	Dehydrogenase activity (mg TPF kg^{-1} h^{-1})				Catalase activity (0.01 M $KMnO_4$ mol L^{-1})			
Treatments	30 days	60 days	120 days	After harvesting	30 days	60 days	120 days	After harvesting
K0W1	14.10	15.30	17.60	12.80	0.25	0.41	0.75	0.12
K0W2	15.50	16.70	19.10	14.10	0.44	0.68	1.14	0.26
K0W3	17.50	18.90	21.40	15.90	0.69	1.02	1.71	0.41
K1W1	21.00	22.50	26.50	18.70	0.87	1.27	2.94	0.54
K1W2	23.70	27.80	34.60	21.00	1.26	1.74	3.62	0.86
K1W3	26.30	31.90	40.60	23.40	1.93	2.56	4.77	1.40
K2W1	28.20	34.40	45.90	24.80	2.07	2.73	5.43	1.52
K2W2	32.60	39.50	53.10	27.90	2.81	3.62	6.65	2.14
K2W3	39.50	45.80	62.10	32.00	3.78	4.89	8.27	2.95
K3W1	25.60	27.70	32.30	20.20	1.19	1.55	4.18	0.72
K3W2	28.20	34.10	40.80	22.10	2.10	2.60	5.06	1.55
K3W3	32.10	39.20	48.10	25.40	2.87	3.44	6.25	2.20
LSD (0.05)	1.81	2.76	3.06	2.19	0.23	0.35	0.51	0.29

K0, K1, K2 and K3 = 0, 25, 50 and 75 mg kg^{-1}, W1, W2 and W3 = 40, 55 and 70% of water-holding capacity, respectively, LSD: Least significant difference

Table 4: Urease and alkaline phosphatase activities in wheat rhizosphere under different rates of potassium fertilizer and water regimes

	Urease (mg NH_4 kg^{-1} h^{-1})				Phosphatase (mg pNP kg^{-1} h^{-1})			
Treatments	30 days	60 days	120 days	After harvesting	30 days	60 days	120 days	After harvesting
K0W1	2.35	3.19	4.24	3.12	201.0	214.0	235.0	178.00
K0W2	3.11	4.08	5.36	3.54	209.0	223.0	249.0	183.00
K0W3	4.29	5.44	6.89	4.15	222.0	245.0	278.0	192.00
K1W1	5.24	6.52	8.16	4.76	230.0	273.0	300.0	209.00
K1W2	6.32	7.71	9.63	5.87	251.0	294.0	333.0	225.00
K1W3	7.56	9.08	11.50	7.04	275.0	323.0	375.0	248.00
K2W1	7.68	9.53	12.40	6.68	281.0	334.0	377.0	242.00
K2W2	9.03	11.30	14.80	7.93	308.0	365.0	423.0	267.00
K2W3	11.10	14.00	18.10	9.59	342.0	402.0	478.0	206.00
K3W1	6.72	8.17	10.60	5.20	243.0	288.0	321.0	214.00
K3W2	8.13	9.77	12.70	6.42	257.0	313.0	359.0	236.00
K3W3	9.65	11.60	15.10	7.71	292.0	353.0	420.0	263.00
LSD (0.05)	0.48	0.74	0.91	0.59	9.2	14.9	19.6	8.42

K0, K1, K2 and K3 = 0, 25, 50 and 75 mg kg^{-1}, W1, W2 and W3 = 40, 55 and 70% of water-holding capacity, respectively, LSD: Least significant difference

Table 5: Microbial biomass carbon and nitrogen (mg kg^{-1}) in wheat rhizosphere under different rates of potassium fertilizer and water regimes

	Microbial biomass C				Microbial biomass N			
Treatments	30 days	60 days	120 days	After harvesting	30 days	60 days	120 days	After harvesting
K0W1	233.0	254.0	278.0	210.0	50.40	56.30	81.70	45.80
K0W2	240.0	263.0	291.0	216.0	54.50	60.90	88.40	49.30
K0W3	255.0	282.0	320.0	229.0	60.80	67.70	98.20	55.10
K1W1	286.0	319.0	365.0	257.0	67.20	71.70	104.00	61.20
K1W2	304.0	342.0	396.0	273.0	74.60	79.70	116.00	68.30
K1W3	336.0	383.0	448.0	302.0	84.40	89.90	130.00	77.60
K2W1	370.0	427.0	508.0	331.0	90.10	96.00	139.00	82.90
K2W2	410.0	480.0	582.0	367.0	102.00	109.00	158.00	94.50
K2W3	456.0	541.0	669.0	409.0	118.00	126.00	182.00	110.00
K3W1	331.0	365.0	402.0	314.0	72.90	80.50	112.00	69.70
K3W2	358.0	399.0	445.0	335.0	82.20	92.80	126.00	78.30
K3W3	392.0	444.0	498.0	368.0	95.00	106.00	141.00	90.40
LSD (0.05)	14.3	16.8	20.9	9.5	5.11	5.97	7.02	4.63

K0, K1, K2 and K3 = 0, 25, 50 and 75 mg kg^{-1}, W1, W2 and W3 = 40, 55 and 70% of water-holding capacity, respectively, LSD: Least significant difference

Table 6: Total bacterial and fungal counts in wheat rhizosphere at different rates of potassium fertilizer and water regimes

	Total bacteria ($\times 10^{10}$ CFU g^{-1})				Total fungi ($\times 10^{3}$ CFU g^{-1})			
Treatments	30 days	60 days	120 days	After harvesting	30 days	60 days	120 days	After harvesting
K0W1	3.11	3.47	4.65	2.35	5.74	6.39	8.57	5.18
K0W2	3.49	3.98	5.49	2.68	5.91	6.64	9.13	5.31
K0W3	3.92	4.58	6.77	3.11	6.19	7.05	9.92	5.70
K1W1	5.40	6.17	8.49	4.48	7.55	8.53	11.70	6.65
K1W2	6.03	6.90	9.60	4.97	8.01	9.09	12.40	7.02
K1W3	7.28	8.31	11.30	6.01	9.03	10.40	14.40	7.86
K2W1	6.60	7.83	10.30	5.54	8.42	9.88	13.90	7.29
K2W2	7.63	9.18	12.10	6.42	9.26	11.10	16.10	7.88
K2W3	9.25	11.10	14.50	7.86	11.20	13.50	19.20	8.91
K3W1	6.24	6.69	8.18	5.29	8.54	8.91	11.90	7.30
K3W2	7.00	7.58	8.73	5.91	9.14	9.69	12.90	7.84
K3W3	8.46	9.25	10.60	7.28	10.20	10.80	14.30	8.75
LSD (0.05)	0.41	0.54	0.89	0.37	0.64	0.79	0.86	0.28

K0, K1, K2 and K3 = 0, 25, 50 and 75 mg kg^{-1}, W1, W2 and W3 = 40, 55 and 70% of water-holding capacity, respectively, LSD: Least significant difference

quality (Kizilkaya and Hepsen, 2007; Garcia-Ruiz *et al*., 2008). Dehydrogenase could reflect the oxidative activity of soil microorganisms and played a central role in the decomposition of organic matters through transferring electrons and protons from substrates to acceptors (Dick *et al*., 1996).

Catalase was also an intracellular enzyme that played a key function in microbial oxidoreductase metabolism and usually found in all aerobic bacteria (Garcia-Gil *et al*., 2000; Wlodarczyk *et al*., 2001; Gianfreda and Ruggiero, 2006). Measurements of soil hydrolyzes enzymes such as urease and phosphatase were important and provided early suggestions of soil fertility due to their relations to mineralization of soil nutrients such as N, P and C. Soil phosphatases was an imperative enzyme in soil P cycling through mineralizing organic P into inorganic P (Pant *et al*., 1999; Gil-Sotres *et al*., 2005). In our study, increases of water regimes from W1 to W3 and K levels from K0 to K3 caused noticeable enhancements in values of the above motioned biological properties. Influence of K was higher than that of the water regime on the chosen biological properties. The effect of K2 and W3 on enzyme activities, microbial biomass and microbial counts was larger than those of other K and water regime levels. Under no K addition, there were no significant differences between W1 and W2. Increasing water regime to W3 led to clear effects on enzyme activities and MBC and MBN at K0. Change of the water regime from W1 to W2 and W3 was responsible for marked increases in biological properties when the soil received K at rates of K1, K2 and K3. These results confirmed the positive roles of K fertilization and water regime in improving soil health indicators. Addition of K at a level of K3 did not show greater effects on biological properties as compared with K2 but its influence was better than K0 and sometimes higher than K1. Using W3 at K1 was generally led to higher values in biological properties than addition of W1 at K4. This indicated that supplying a good water level to the soil could have larger effects on biological properties than addition of K at a high dose. Comparing between the studied treatments, using K2W3 treatment led to largest values of urease (18.1), catalase (8.27), phosphatase (478), dehydrogenase (62.1), MBC (669), MBN (182), bacteria (14.5×10^{10}) and fungi (19.2×10^{3}) after 120 days. The lowest values of enzyme activities, microbial biomass and microbial counts that noticed at W1 might be resulted from low releasing, transferring and diffusion of soil nutrients. Our results are in agreement with Ibrahim *et al*. (2015), who showed in an incubation experiment to evaluate the effect of different water levels on MBC that addition 70% of WHC caused higher MBC values as compared with 40 and 55% of WHC. Addition of K had a vital role in simulating soil microorganisms and this explained the larger improvements in soil biological properties. Ahamadou *et al*. (2009) showed that addition of K fertilizer increased MBC and MBN values from 97.57 and 28.53 mg kg^{-1} to 283.9 and 38.02 mg kg^{-1}, respectively. The lowest values of enzyme activities, microbial biomass and microbial counts were determined the harvest of wheat, whereas the highest values were noticed after 120 days of wheat thinning. So, it is interesting to mention that temperature had major effects on soil biological properties. It was detected by Iqbal *et al*. (2010) and Hassan *et al*. (2015) that the increase of temperature degree led to significant variations in soil biological properties such as MBC, MBN, catalase and total microbial counts (bacteria and fungi). The smallest values of soil biological properties that detected at the harvest of wheat in our study were probably resulted from the decrease of soil organic matter and different nutrients releasing as well as root exudates at plant maturity stage.

CONCLUSION

In conclusion, the results of our study clearly demonstrated that changes of water regime and K rates had marked effects on soil microbial properties. The medium dose of K (K2 = 50 mg kg^{-1}) at W3 (70% of WHC) was responsible for the highest values of cumulative CO_2, microbial biomass carbon and nitrogen and enzyme activities. Therefore, managements of water and K additions

should be considered as good practices to improve the quality of clayey soils in Egypt. Further studies are needed to evaluate the efficiency of water application and K fertilizers at different rates under field conditions on growth and productivity of plants.

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