

Full Length Research Paper

# The effects of boron management on soil microbial population and enzyme activities

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**Boron is an essential micronutrient required for plant growth. Soil microorganisms directly influence boron content of soil as maximum boron release corresponds with the highest microbial activity. The objective of this study is to determine the effects of different levels of boron fertilizer on microbial population, microbial respiration and soil enzyme activities in different soil depths in cultivated wheat soils. A randomized block design with three replications was used in this experiment. Field experiments were conducted to evaluate the effects of B levels (0, 1, 3, 6 and 9 kg ha<sup>-1</sup> B) on soil microbial population in cultivated wheat (*Triticum vulgare* cultivar Dogu-88) soils. Statistical results showed a significant ( $p < 0.01$ ) differences between B applications and microbial population and between B applications and microbial respiration in 0 to 30 and 30 to 60 cm soil depths. The highest population of bacteria, fungi, actinomycetes and CO<sub>2</sub>-C production were observed at 3 kg ha<sup>-1</sup> B level in different growing periods of the plant and in different soil depths. Urease, phosphatase and dehydrogenase enzyme activities showed a significant ( $p < 0.01$ ) positive correlation with B applications. The highest urease activity was observed in 6 kg ha<sup>-1</sup> B level and the highest phosphatase and dehydrogenase enzyme activities were observed in 3 kg ha<sup>-1</sup> B level in harvest period in both soil depths.**

**Key words:** Boron management, soil microbial population, urease activity, phosphatase activity, dehydrogenase activity.

## INTRODUCTION

Boron (B) deficiency has been reported in 132 crops in 80 countries (Shorrocks, 1997) and is a major cause of crop yield loss in China, India, Nepal and Bangladesh (Anantawiroon et al., 1997). In Turkey, B deficiency was

identified through individual field trials (Gezgin and Hamurcu, 2006) and micronutrient availability studies (Gezgin et al., 1999). It is estimated that in the central southern and eastern Anatolia regions of Turkey, 27 to 34% of the soils are B-deficient (Gezgin and Hamurcu, 2006; Angin et al., 2008).

Boron management is challenging because the optimum B application range is narrow and optimum B application rates can differ from one soil to another Gupta, 1993; Marschner, 1995). The boron content in the soil changes between 2 and 100 ppm (Swaine, 1955). Average boron is considered 30 ppm in soil depending on the main rock; boron content in the soil exhibits a large variation. Consequently, plants need trace amounts of boron but it becomes toxic at 2 ppm or greater for most plants (Carlos, 2000). A tolerable boron concentration for plants in soils is approximately 25 ppm (Khan 2009). Generally speaking, there is more boron in the subsoil

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**Abbreviations:** IAA, Indole acetic acid; loss-on-ignition; DTPA, diethylenetriaminepentaacetic acid; SEA, soil extract agar; DPA, dextrose-peptone agar; RBME, rose bengal-malt extract agar; CFU, colony forming units; ODE, oven-dried equivalent; BR, basal respiration; WFP, water-filled porosity; UE, urease enzyme; Alk-P, alkaline phosphatase; pNPP, para-nitrophenyl phosphate; DH, dehydrogenase; TTC, tetrazolium chloride; IAA, indole acetic acid; PBS, phosphate buffered saline; TPF, triphenyl formazone.

and deeper (Haas, 1992).

The essentiality of B for growth and development of higher plants has been earlier demonstrated (Marschner, 1995; Shelp, 1993). Boron plays an important role in the movement and metabolism of carbohydrate in the plant, synthesis of plant hormones and nucleic acids, pollen germination, flowering and fruiting, water use, nitrogen assimilation and generative growth of plants. It also functions in lignin formation of cell wall synthesis, cell division, differentiation, membrane functioning, root elongation and salt absorption (Haas, 1992; Marschner, 1995; Simmons, 1998; Anonymous 2007). The most important functions of boron in plants are thought to be its structural role in cell wall development and stimulation or inhibition of specific metabolism pathways (Ahmad et al., 2009). Loomis and Durst (1992) reported that, boron is an essential micronutrient required for growth and development of vascular plants, diatoms and species of marine algal flagellates, while bacteria, fungi, green algae and animals apparently do not require boron. Leguminous plants as well as cyanobacteria require B for N<sub>2</sub> fixation, as B plays a major role in nitrogen assimilation (Brown and Shelp, 1997).

Boron availability is dependent on several factors existing in the soil-plant system such as soil organic matter, soil texture, cultivation, soil moisture, temperature, soil pH and liming, soil fertility and microbial activity (Anonymous, 1995). Microorganisms break down soil organic matter, which allows the release of boron from organic complexes. Microbial activity is lowest under drought conditions or in cold wet soils. It is highest when soils are moist and warm. Where microbial activity is highest, boron release is highest (Anonymous, 1995). Boron in the soil, an important fraction is associated with organic matter and is released through microorganism activities (Berger and Pratt, 1963). By using the nutrient themselves and then, contributing their bodies to the soil's fertility load, microorganisms change boron into an organic form (Haas, 1992).

The effects of B deficiency, sufficiency and toxicity on the mineral nutrient content of plants are not well established (Tariq and Mott, 2007). The range between deficient and toxic B concentration is smaller than for any other nutrient element (Goldberg, 1997). Boron deficiency reduces growth of soil bacteria and poor movement of sugar and carbohydrates in the plant, wall synthesis, lignification, cell wall structure, carbohydrate metabolism, RNA metabolism, respiration, indole acetic acid (IAA) metabolism, phenol metabolism and membrane integrity (Pollard et al., 1977; Anonymous 2008). High concentrations of boron in soils cause toxicity and promote diseases and enzyme problems. Only in a few cases did the number of ammonifying bacteria and microscopic fungi as well as the enzyme activity increase (Kolesnikov et al., 2008). Crop response to B application has been documented for wheat (*Triticum durum* Desf.) (Soylu et al., 2004), sunflower (*Helianthus annuus* L.) (Asad et al.,

2002; Oyinlola, 2007), and chickpea (*Cicer arietinum* L.) (Ceyhan et al., 2007). Gupta (1971) noted that, barley was more sensitive than wheat and could not stand even 0.5 ppm added boron. Wheat has a low boron demand. Wilcox (1960) suggested that, wheat and barley belonged to semi-tolerant category and could not stand more than 2.0 ppm B in irrigation waters. Manchanda and Yadav (1978) have shown that, barley grain yield was significantly increased up to 3 ppm B level, but decreased with higher B levels.

Boron is essential in regulation of enzymatic processes in plants and Alvarez-Tinaut (1990) observed positive correlations between B, Fe and Cu contents of sunflower and suggested that boron could indirectly affect catalase activity via Fe and Cu. Positive correlation between Zn and B, also indicates that B could indirectly affect enzymatic processes through modification of the Zn content. According to Hu and Brown (1994), the stimulatory effect of B on H<sup>+</sup> ATPase activity requires the presence of auxin or enhancement in proton release by auxin requires the presence of boron. Cakmak and Romheld (1997) stated that, a higher amount of phenolic compounds is accumulated in B-deficient plants. According to Cakmak (1994), in these plants, not only the phenolic compounds increase but also defense capacity of cells against toxic O<sub>2</sub> species is weakened due to reduced levels of ascorbic acid, SH-compounds and H<sub>2</sub>O<sub>2</sub> scavenging enzymes.

Despite such influence of B on plant growth and function, no evidence has yet been presented to show that B is an enzyme constituent or it has a direct role in enzyme activities. It is also not clear whether these processes are precursor of direct functions of B or the changes are of an indirect nature. Therefore, further research is necessary to have an insight on the role of boron in plant growth and soil functions. Few studies have looked at the direct effect of B fertilizer on soil microbial properties and enzyme activities.

The objective of this study is to determine the effects of different levels of B fertilizers on microbial population (bacteria, fungi and actinomycetes), microbial respiration (CO<sub>2</sub>-C production) and enzyme activities (urease, phosphatase and dehydrogenase) of soils that are being cultivated with wheat.

## MATERIALS and METHODS

### Experimental site

This study was conducted at the Agricultural Research Station of Ataturk University located in Erzurum, Turkey (lat. 41°16' E, long. 39°55' N) during the summer (late May to late September) of 2007. The altitude of the experiment station is 1835 m and prevailing semiarid climate. During the growing period in 2007, the minimum and maximum temperature was 11.4 and 26.5°C, respectively. The mean relative humidity, wind speed, daily sunshine, total precipitation and total evaporation were 59.52%, 2.74 m s<sup>-1</sup>, 11.18 h, 62.6 and 391.2 mm in 2007 (1 May to 29 September), respectively. The predominant soil was an Aridisol with parent materials mostly consisting of volcanic, marn and lacustrin transported

material (Soil Survey Staff, 1999).

### Experimental design

The experiment was conducted following a randomized block design with different levels of B fertilizer as subplot treatment in triplicate. Winter wheat (*T. vulgare* cultivar Dogu-88) was sown in October 2006, at a seeding rate of 165 kg ha<sup>-1</sup> with 22 cm row spacing, using a combined fertilizer and seed drill. Fertilizer application rates were based on standard soil tests conducted by the Ataturk University Soil Testing Laboratory. Before planting, N, P, K and B were broadcast in each plot at the rates of 300 kg (150 kg at sowing and 150 kg in May 2007) N ha<sup>-1</sup> as ammonium nitrate (33% N), 95 kg ha<sup>-1</sup> P as diamonium phosphate (18% N, 46% P<sub>2</sub>O<sub>5</sub>), 150 kg ha<sup>-1</sup> K as potassium sulfate (50% K<sub>2</sub>O), and 0, 1, 3, 6 and 9 kg ha<sup>-1</sup> Borax (11% B) (as Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O), respectively (Booij 2000; Turan and Sevimli, 2005). The crop was weeded manually, as required with a hoe and was harvested in September 2007.

### Soil sampling and laboratory analysis techniques

Before sowing initial soil, samples were collected for analysis of some chemical and physical properties. At sowing (October 2006), flowering (August 2007) and harvest period (September 2007) soil samples were collected for analysis of microbial population, microbial respiration and enzyme activities of soils. All soil samples taken from 0 to 30 and 30 to 60 cm depths were composited and sieved through a 2 mm mesh. After sieving, total organic matter (SOM) content was determined by following the standard loss-on-ignition (LOI) method (Nelson and Sommers, 1996). The CaCO<sub>3</sub> content was determined by using the pressure calcimeter method (Leoppert and Suarez, 1996). Total N content of soil was measured by the micro-Kjeldahl method (Bremner, 1996). Soil pH was determined by using a glass electrode meter in 1:2.5 soil:water ratio (Handershot et al., 1993). Effective cation exchange capacity was calculated as the sum of exchangeable cations (Sumner and Miller, 1996). Exchangeable cations were determined by atomic absorption spectrophotometer (Harlon and DeVore, 1989). The available P in soil was determined by following ammonium molybdate-ascorbic acid method (Knudsen and Beegle, 1988). Microelements in the soils were determined by diethylenetriaminepentaacetic acid (DTPA) extraction method (Lindsay and Norvell, 1978). Boron was determined using the azomethine-H extraction as described in Wolf (1974) and an Aquamat ultraviolet/VIS spectrophotometer (Thermo Electron Spectroscopy Ltd., Cambridge, U.K.). Soil particle size distribution (sand, silt and clay content) was determined by the hydrometer method (Gee and Bauder, 1986). Soil textural class was determined by following the USDA textural triangle. Electrical conductivity was measured in saturation extracts according to Rhoades (1996). Selected soil physical and chemical properties are given in Table 1.

### Soil biological analysis

Culturable bacteria, fungi and actinomycetes cells were enumerated by using spread soil dilution plate method. For bacteria, fungi and actinomycetes, each dilution of the series up to 10<sup>6</sup> and 10<sup>7</sup> in phosphate buffered saline (PBS) was prepared and spread onto Petri-dishes. Soil extract agar (SEA) was used for bacterial incubation at 30°C for 7 days, dextrose-peptone agar (DPA) was used for fungal incubation at 25°C for 7 days and rose bengal-malt extract agar (RBME) was used for actinomycetes incubation at 28°C for 14 days (Cynathia, 2003). After the incubation, the

average colony forming units (CFU) per gram of oven-dried equivalent (ODE) of field-moist soil was calculated by using an automated colony counter (Canbolat et al., 2006; Madigon et al., 2006).

Basal respiration (BR), as a measure of soil biological activity, was determined by using *in vitro* static incubation of unamended field-moist soil (Islam and Weil, 2000). About 20 g ODE of field-moist soil adjusted at 70% water-filled porosity (WFP) was taken in 25 ml glass beakers. Each soil sample was placed in a 1 L mason jar along with a glass vial containing 10 ml of distilled deionized water to maintain humidity and a plastic vial containing 10 ml of 0.5 M NaOH to trap CO<sub>2</sub> evolved from the incubated soil. The mason jars were sealed airtight and incubated in the dark at 25 ± 1°C for 20 days. The CO<sub>2</sub> evolved over time was absorbed in the 0.5 M NaOH followed by precipitation as BaCO<sub>3</sub> by the addition of excess 1 M BaCl<sub>2</sub>. The remaining NaOH in each vial was then titrated to the phenolphthalein endpoint with a standardized 0.5 M HCl solution. The BR rate was calculated as below:

$$\text{BR rates (mg CO}_2\text{/kg soil)} = (\text{CO}_2\text{soil} - \text{CO}_2\text{air})/20 \text{ days}$$

Urease enzyme (UE) activity was assayed by using urea solution and expressed as µg NH<sub>4</sub>-N per g soil and incubation time (2 h). The activities of alkaline phosphatase (Alk-P) were assayed by using para-nitrophenyl phosphate (pNPP) and expressed as I µg pNPP per gram soil and incubation time (hours). Dehydrogenase (DH) activity was assayed using triphenyl tetrazolium chloride (TTC) and expressed as µg triphenyl formazone (TPF) per gram soil and incubation time (24 h) according to Tabatabai (1994).

### Statistical analysis

Statistical analysis for soil microbial population and CO<sub>2</sub>-C production were used for repeated measures of analysis of variance (ANOVA). Comparison of means was performed when the F-test for treatment was significant at the 5% level using Duncan's multiple means tests at a predetermined level of *p* < 0.05. In addition, we evaluated the impact of different levels of B application on soil enzyme activities by calculating regression equation and simple linear correlation coefficient (*r*) procedures. The Statistical Package for the Social Sciences (SPSS) 17.0 package was used for all statistical analysis.

## RESULTS

Some physical and chemical analyses of the soil are given in Table 1. Most soils had alkaline reaction and contained sufficient N and organic matter content ranging from 0.6 to 1.8%. The soils did not exhibit any salinity problem but there was enough lime present in the soil to be classified calcareous soils. There were sufficient Ca and K in the soils. However, the availability of P and Mg was slightly less than optimum level (FAO 1990).

### Effects of boron application on microbial population and CO<sub>2</sub>-C production

The quantitative analyses of soil bacteria, fungi, actinomycetes and CO<sub>2</sub>-C production under different levels of B application are presented in Table 2. According to the results of ANOVA, the effects of B fertilization on

**Table 1.** Some chemical and physical properties of the experimental soils before sowing.

Soil property	Soil depth		
	0 to 30 cm	30 to 60 cm	
pH (1:2 soil: water)	7.25	10.45	
Organic matter g kg <sup>-1</sup>	18	6.65	
CaCO <sub>3</sub> g kg <sup>-1</sup>	150	137.28	
Total N g kg <sup>-1</sup>	0.78	0.28	
Available P mg kg <sup>-1</sup>	9.3	3.28	
Cation exchangeable capacity cmolc kg <sup>-1</sup>	32.0	27.5	
Exchangeable cations, cmolc kg <sup>-1</sup>	Ca <sup>+2</sup>	19.8	20.09
	Mg <sup>+2</sup>	4.5	3.04
	K <sup>+1</sup>	2.7	1.33
	Na <sup>+1</sup>	0.15	0.10
Microelements, mg kg <sup>-1</sup>	Fe <sup>+2</sup>	3.36	1.32
	Mn <sup>+2</sup>	1.74	1.17
	Zn <sup>+2</sup>	1.75	1.14
	Cu <sup>+2</sup>	0.78	0.69
	B	0.16	0.08
Electric conductivity dS m <sup>-1</sup>	1.44	1.05	
Soil particle size distribution	Clay %	32.6	27.5
	Silt %	42.3	40.4
	Sand %	25.1	32.1
Soil textural class	CL	L	

the average number of bacteria, fungi and actinomycetes were statistically significant ( $p < 0.01$ ) in all growing periods and in both soil depths. Similarly, statistical results showed a significant ( $p < 0.01$ ) differences between B application levels-plant growing periods and B application levels-soil depths.

Microbial population increased up to 3 kg ha<sup>-1</sup> B level, which was significantly higher than control in 0 to 30 cm and 30 to 60 cm soil depths. The highest average number of bacteria were observed in 3 kg ha<sup>-1</sup> B application in 0 to 30 and 30 to 60 cm soil depths in sowing, flowering and harvest periods (34.67, 15.27x10<sup>6</sup> CFU g<sup>-1</sup> soil; 47.50, 24.15x10<sup>6</sup> CFU g<sup>-1</sup> soil; 37.60, 21.05x10<sup>6</sup> CFU g<sup>-1</sup> soil, respectively). The number of bacteria decreased at B levels more than 3 kg ha<sup>-1</sup> B levels in all growing periods and at both soil depths. The highest average bacteria numbers were observed in flowering period in two soil depths (40.93, 21.11x10<sup>6</sup> CFU g<sup>-1</sup> soil) (Table 2).

According to Table 2, the highest number of fungi were also observed in 3 kg ha<sup>-1</sup> B level and in all growing period of plant in both soil depths (46.91 and 65.21x10<sup>4</sup>

CFU g<sup>-1</sup> soil; 72.64, 95.80x10<sup>4</sup> CFU g<sup>-1</sup> soil; 61.25, 79.70x10<sup>4</sup> CFU g<sup>-1</sup> soil, respectively). The highest average number of fungi was observed in flowering period in both soil depths (62.52, 83.15x10<sup>6</sup> CFU g<sup>-1</sup> soil).

Similar observations were also made for actinomycetes with the highest average number at 3 kg ha<sup>-1</sup> B application in 0 to 30 and 30 to 60 cm soil depths in all growing periods (21.00, 35.79x10<sup>3</sup> CFU g<sup>-1</sup> soil; 31.03, 55.07x10<sup>3</sup> CFU g<sup>-1</sup> soil; 24.19, 43.36x10<sup>3</sup> CFU g<sup>-1</sup> soil, respectively). The number of actinomycetes decreased at higher levels of B addition in all growing periods and at both depths. The highest average number of actinomycetes was observed in flowering period in the two soil depths (24.03, 46.73x10<sup>6</sup> CFU g<sup>-1</sup> soil). In general, bacterial population was more abundant in 0 to 30 than in 30 to 60 cm soil depth. However, the fungi and actinomycetes population were found lower in 0 to 30 cm than in 30 to 60 cm soil depth (Table 2).

Soil respiration was significantly ( $p < 0.01$ ) affected both by soil depths and the different levels of B fertilizer. The highest CO<sub>2</sub>-C production was observed in 3 kg ha<sup>-1</sup>

**Table 2.** Effect of boron applications on soil microbial population and CO<sub>2</sub>-C production.

Soil Depth (cm)	B Rate (kg da <sup>-1</sup> )	Microbial population									CO <sub>2</sub> -C production		
		Bacteria			Fungi			Actinomycetes			CO <sub>2</sub> -C		
		SP	FP	HP	SP	FP	HP	SP	FP	HP	SP	FP	HP
0-30	0	25.70 <sup>b</sup>	37.75 <sup>b</sup>	29.61 <sup>ab</sup>	36.16 <sup>b</sup>	57.03 <sup>b</sup>	46.77 <sup>b</sup>	16.99 <sup>b</sup>	22.25 <sup>b</sup>	16.65 <sup>b</sup>	6.48 <sup>b</sup>	9.94 <sup>ab</sup>	7.78 <sup>b</sup>
	1	31.42 <sup>ab</sup>	42.00 <sup>ab</sup>	30.36 <sup>ab</sup>	42.75 <sup>ab</sup>	67.30 <sup>ab</sup>	53.26 <sup>ab</sup>	17.33 <sup>ab</sup>	25.19 <sup>ab</sup>	19.85 <sup>ab</sup>	7.79 <sup>ab</sup>	11.52 <sup>ab</sup>	8.40 <sup>ab</sup>
	3	34.67 <sup>a</sup>	47.50 <sup>a</sup>	37.60 <sup>a</sup>	46.91 <sup>a</sup>	72.64 <sup>a</sup>	61.25 <sup>a</sup>	21.00 <sup>a</sup>	31.03 <sup>a</sup>	24.19 <sup>a</sup>	8.57 <sup>a</sup>	12.67 <sup>a</sup>	10.20 <sup>a</sup>
	6	25.37 <sup>ab</sup>	47.00 <sup>a</sup>	24.80 <sup>b</sup>	40.48 <sup>ab</sup>	72.00 <sup>a</sup>	59.12 <sup>a</sup>	17.66 <sup>ab</sup>	26.95 <sup>ab</sup>	20.46 <sup>ab</sup>	7.33 <sup>ab</sup>	12.55 <sup>a</sup>	8.26 <sup>ab</sup>
	9	13.34 <sup>b</sup>	30.40 <sup>c</sup>	16.90 <sup>c</sup>	25.44 <sup>c</sup>	43.63 <sup>c</sup>	35.68 <sup>c</sup>	11.66 <sup>c</sup>	14.71 <sup>c</sup>	11.43 <sup>c</sup>	4.96 <sup>c</sup>	7.70 <sup>b</sup>	4.94 <sup>c</sup>
Ave.		26.10 <sup>b</sup>	40.93 <sup>a</sup>	27.85 <sup>b</sup>	38.35 <sup>c</sup>	62.52 <sup>a</sup>	51.22 <sup>b</sup>	16.93 <sup>b</sup>	24.03 <sup>a</sup>	18.52 <sup>b</sup>	7.03 <sup>b</sup>	10.88 <sup>a</sup>	7.92 <sup>b</sup>
30-60	0	13.20 <sup>b</sup>	19.27 <sup>b</sup>	15.03 <sup>ab</sup>	52.10 <sup>b</sup>	76.29 <sup>b</sup>	59.67 <sup>b</sup>	25.93 <sup>b</sup>	42.39 <sup>b</sup>	33.19 <sup>b</sup>	2.71 <sup>b</sup>	4.15 <sup>b</sup>	3.42 <sup>b</sup>
	1	14.36 <sup>a</sup>	23.56 <sup>a</sup>	16.81 <sup>ab</sup>	60.14 <sup>ab</sup>	89.12 <sup>ab</sup>	63.98 <sup>ab</sup>	32.09 <sup>a</sup>	49.65 <sup>ab</sup>	36.81 <sup>ab</sup>	3.12 <sup>a</sup>	5.05 <sup>a</sup>	4.02 <sup>ab</sup>
	3	15.27 <sup>a</sup>	24.15 <sup>a</sup>	21.05 <sup>a</sup>	65.21 <sup>a</sup>	95.80 <sup>a</sup>	79.70 <sup>a</sup>	35.79 <sup>a</sup>	55.07 <sup>a</sup>	43.36 <sup>a</sup>	3.67 <sup>a</sup>	5.60 <sup>a</sup>	5.75 <sup>a</sup>
	6	14.00 <sup>a</sup>	24.00 <sup>a</sup>	19.79 <sup>a</sup>	57.37 <sup>ab</sup>	95.00 <sup>a</sup>	64.38 <sup>ab</sup>	29.93 <sup>ab</sup>	54.50 <sup>a</sup>	38.03 <sup>ab</sup>	3.23 <sup>a</sup>	5.21 <sup>a</sup>	3.92 <sup>ab</sup>
	9	8.34 <sup>c</sup>	14.57 <sup>c</sup>	11.23 <sup>b</sup>	39.02 <sup>c</sup>	59.54 <sup>c</sup>	39.36 <sup>c</sup>	18.89 <sup>c</sup>	32.02 <sup>c</sup>	21.29 <sup>c</sup>	2.45 <sup>b</sup>	3.29 <sup>b</sup>	2.74 <sup>c</sup>
Ave.		13.03 <sup>c</sup>	21.11 <sup>a</sup>	16.78 <sup>b</sup>	54.77 <sup>c</sup>	83.15 <sup>a</sup>	61.42 <sup>b</sup>	28.52 <sup>c</sup>	46.73 <sup>a</sup>	34.54 <sup>b</sup>	3.04 <sup>c</sup>	4.66 <sup>a</sup>	3.97 <sup>b</sup>

SP, Sowing period; FP, flowering period; HP, harvest period. Bacteria 10<sup>6</sup> CFU g<sup>-1</sup> soil; Fungi 10<sup>4</sup> CFU g<sup>-1</sup> soil; Actinomycetes 10<sup>3</sup> CFU g<sup>-1</sup> soil, CO<sub>2</sub>-C mg C m<sup>-2</sup> h<sup>-1</sup>.

B application level in both soil depths and for all growing periods (8.57, 3.67x10<sup>3</sup> CFU g<sup>-1</sup> soil; 12.67, 5.60x10<sup>3</sup> CFU g<sup>-1</sup> soil; 10.20, 5.75x10<sup>3</sup> CFU g<sup>-1</sup> soil, respectively). Average CO<sub>2</sub>-C production increased with B application up to 3 kg ha<sup>-1</sup> level but decreased at levels higher than 3 kg ha<sup>-1</sup> B in all growing periods. The highest average CO<sub>2</sub>-C production was observed in flowering period for both soil depths (10.88, 4.66x10<sup>6</sup> CFU g<sup>-1</sup> soil) with higher CO<sub>2</sub>-C fluxes in 0.30 cm soil depth (Table 2).

#### Effects of boron application on some soil enzyme activities

We also monitored the activities of three enzyme

groups; urease, phosphatase and dehydrogenase in different boron fertilizer levels and different depths of the soil. Urease and phosphatase enzyme activities in the soil showed a significantly positive correlation with B applications levels in different plant growing periods and in different soil depths. The lowest urease enzyme activity was observed in 9 kg ha<sup>-1</sup> B application level at sowing period in 0 to 30 cm upper layer (36.12 μg NH<sub>4</sub><sup>+</sup>-N g<sup>-1</sup> soil 2h<sup>-1</sup>) and 0 kg ha<sup>-1</sup> (control) B application level at sowing period in 30 to 60 cm lower layer (19.27 μg NH<sub>4</sub><sup>+</sup>-N g<sup>-1</sup> soil 2h<sup>-1</sup>).

The highest urease activity was observed in 6 kg ha<sup>-1</sup> boron application at harvest period in both soil depths (138.52 μg NH<sub>4</sub><sup>+</sup>-N g<sup>-1</sup> soil 2h<sup>-1</sup>, r<sup>2</sup>= 0.80, p < 0.01; 85.28 μg NH<sub>4</sub><sup>+</sup>-N g<sup>-1</sup> soil 2h<sup>-1</sup>, r<sup>2</sup>= 0.89, p < 0.01, respectively). The urease enzyme

activity increased up to 6 kg ha<sup>-1</sup> B application level and decreased with further addition of B for all growing periods and in two different soil depths. Urease enzyme activities of the soil was observed to be higher in superficial layer than in subsuperficial layer (Figure 1).

The highest phosphatase enzyme activity was observed in 3 kg ha<sup>-1</sup> boron application and harvest period in both soil depths (76.0 μg pNP g<sup>-1</sup> soil h<sup>-1</sup>, r<sup>2</sup>= 0.99, p < 0.01; 41.96 μg pNP g<sup>-1</sup> soil h<sup>-1</sup>, r<sup>2</sup>= 0.99, p < 0.01, respectively). The phosphatase enzyme activity increased up to 3 kg ha<sup>-1</sup> B level and then, decreased with more B additions in all growing periods and in both soil depths (Figure 2).

Similarly, dehydrogenase enzyme activities of the soils also showed a significantly positive

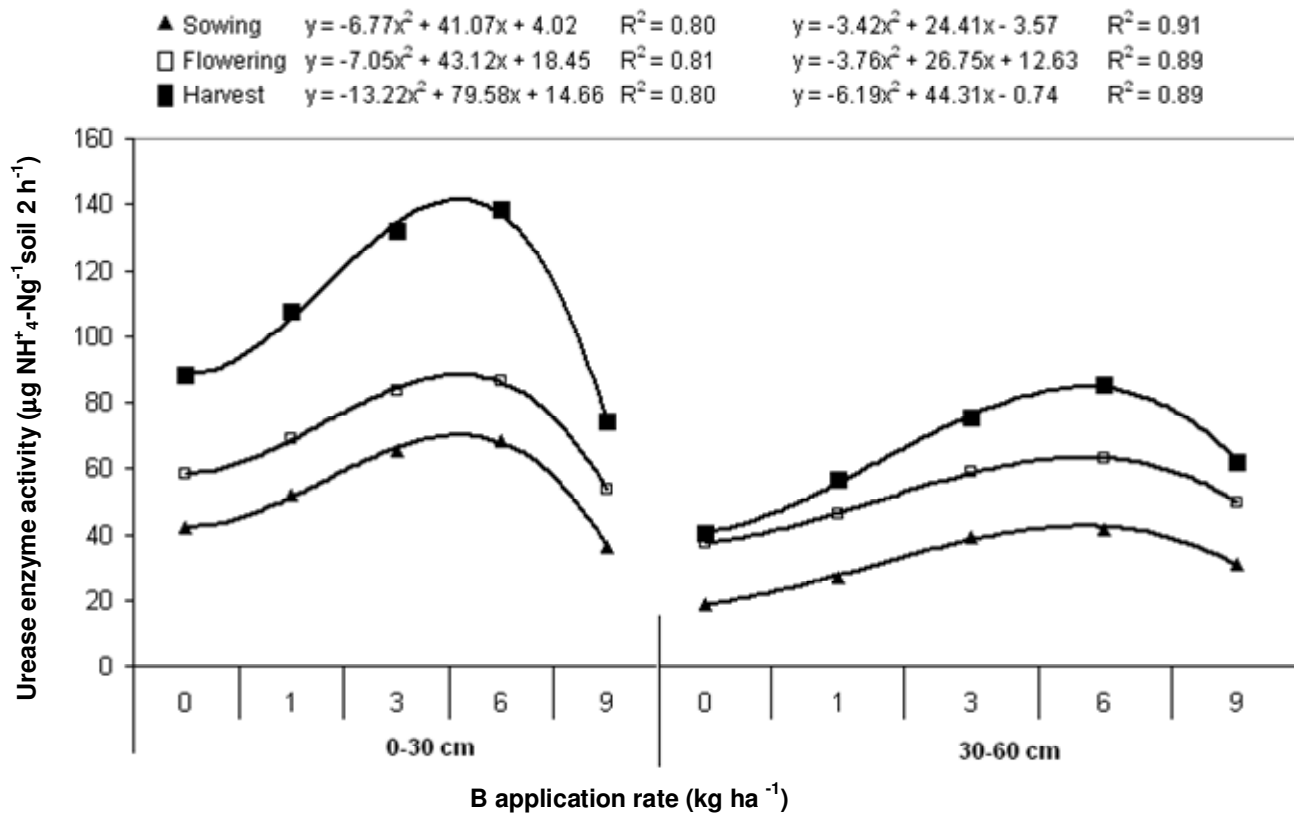


Figure 1. The effects of B application on urease enzyme activities of the soil in different soil depths.

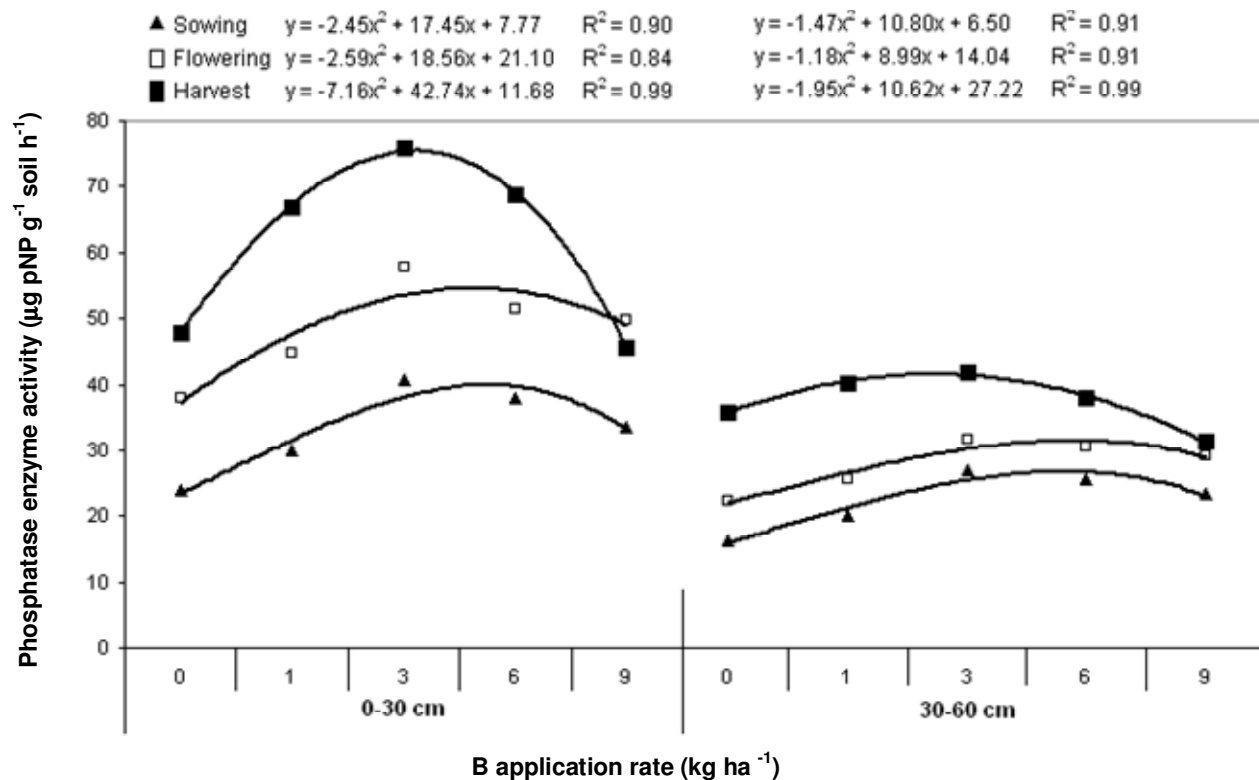
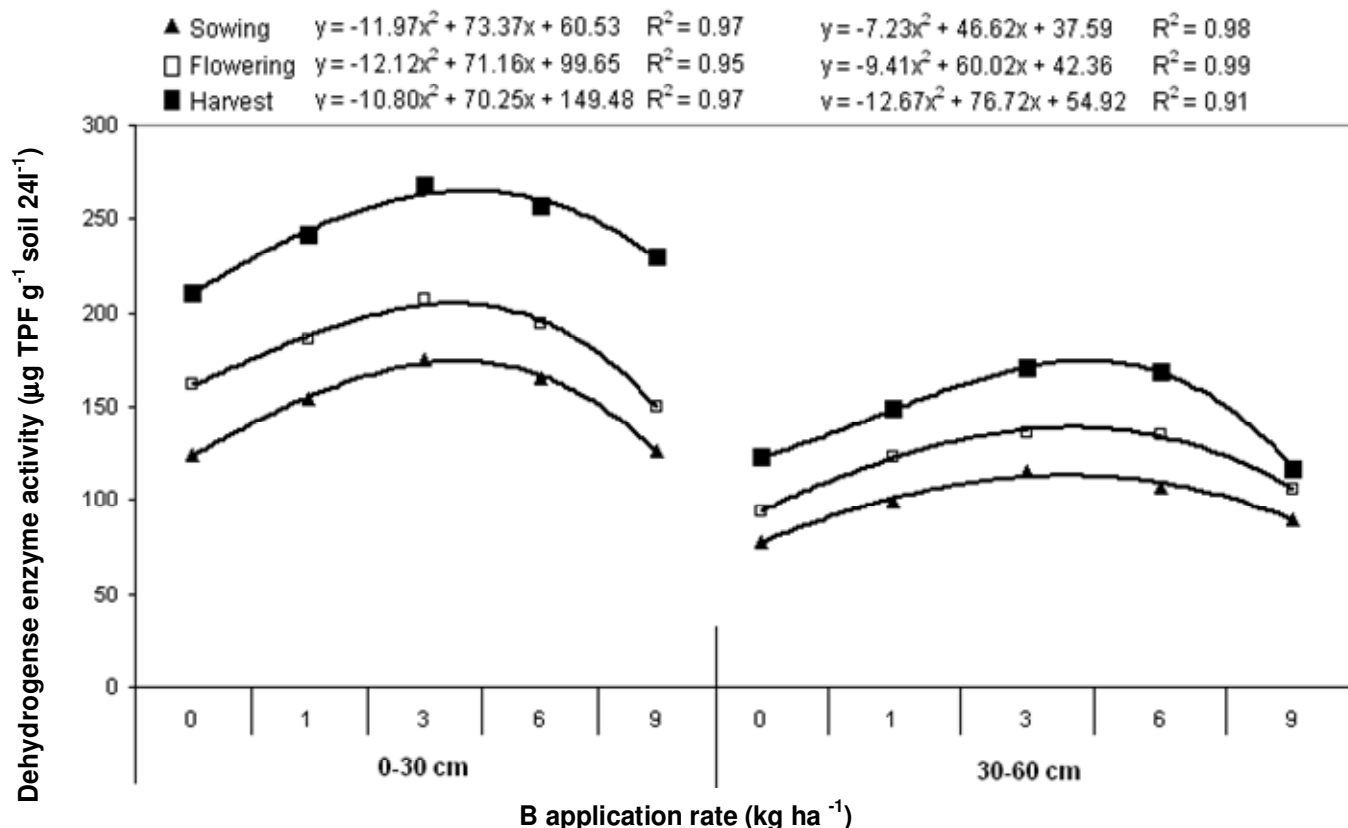


Figure 2. The effects of B application on phosphatase enzyme activities of the soil in different soil depths.



**Figure 3.** The effects of B application on dehydrogenase enzyme activities of the soil in different soil depths.

correlation with B application levels in different growing periods and in different soil depths. The highest dehydrogenase enzyme activity was observed at  $3 \text{ kg ha}^{-1}$  B application level and at harvest period in both soil depths ( $267.31 \mu\text{g TPF g}^{-1} \text{ soil } 24 \text{ h}^{-1}$ ,  $r^2 = 0.97$ ,  $p < 0.01$ ;  $170.90 \mu\text{g TPF g}^{-1} \text{ soil } 24 \text{ h}^{-1}$ ,  $r^2 = 0.99$ ,  $p < 0.01$ , respectively). The lowest was observed in  $0 \text{ kg ha}^{-1}$  (control) boron application and at sowing period in 0 to 30 cm ( $124.23 \mu\text{g TPF g}^{-1} \text{ soil } 24 \text{ h}^{-1}$ ) and 30 to 60 cm ( $77.83 \mu\text{g TPF g}^{-1} \text{ soil } 24 \text{ h}^{-1}$ ) soil depths. The dehydrogenase enzyme activity followed similar trend as alkaline phosphatase, increased up to  $3 \text{ kg ha}^{-1}$  B level and decreased with higher levels of B additions. Urease, phosphatase and dehydrogenase enzyme activities were observed more in the upper layer of the soil profile in comparison with the lower layer in the profile (Figure 3).

## DISCUSSION

Wheat has a low boron demand (Gupta, 1971). Wheat belonged to semi-tolerant category and could not stand more than 2.0 ppm B in irrigation waters (Wilcox, 1960). Total boron content in the soil, an important fraction is associated with organic matter and released through microorganism activity (Berger and Pratt, 1963). Accord-

ing to our results, applied B fertilizer were also helpful in creating a better soil biological environment of cultivated wheat soil and were well evidence in the present study by the increased microbial population and enzymatic activity. The population of bacteria, fungi and actinomycetes significantly increased with B application level. Increasing B application has rapidly changed soil microbial biomass and the biologically active fraction in soil. The availability of readily mineralized C and N and improvement in the physico-chemical properties of the soil might have improved the microbial population of the soil (Baradwaj and Datt, 1995).

Higher levels of B levels over the  $3 \text{ kg ha}^{-1}$  B decreased soil microbial populations and  $\text{CO}_2\text{-C}$  production. High concentrations of B in soils cause toxicity for microorganisms. Only in a few cases did the number of bacteria and microscopic fungi increase (Kolesnikov et al., 2008). Availability and use of soil B depend on fertility levels. Soils with low organic matter will usually need more frequent boron fertilization at lower amounts per hectare (Fleming, 1980). Berger and Pratt (1963) have shown that, from the total boron content in the soil, an important fraction is associated with organic matter and released through microorganism activity. Microorganisms break down soil organic matter, which allows the release of B from organic complexes. Changes in B level also

induce changes in soil CO<sub>2</sub>-C production. Where microbial activity and CO<sub>2</sub>-C production are highest, boron release is highest (Anonymous, 1995). Our results have shown similar results with these studies.

Enzyme activities play key roles in the biochemical functioning of soils (Frankerberger and Dick, 1983; Acosta-Martínez et al., 2007). Phosphatase enzymes can be a good indicator of the organic phosphorus mineralization potential and biological activity of soils (Dick et al., 1983). Dehydrogenase enzymes play a significant role in the biological oxidation of soil organic matter (Dick and Tabatabai, 1993). We observed that, urease and phosphatase enzyme activities showed a significantly positive correlation with boron applications levels in different growing periods and in different soil depths. Depending on increasing B fertilizer level, urease, phosphatase and dehydrogenase enzyme activities of the soil increased. Mineralized C and N improve the physico-chemical properties of the soil and depending on the application of boron the microbial population, enzyme activities of the soil increased (Baradwaj and Datt, 1995). The increase in the soil enzymatic activity may be ascribed to the easily biodegradable organic matter imposed in the soil, which stimulated the growth of soil microorganisms and soil enzyme activities (Perucci, 1992). Higher levels of B levels decreased soil enzyme activities. High concentrations of boron in soils have caused toxicity and promoted enzyme problems (Kolesnikov et al., 2008).

## Conclusions

It has been shown that, increasing B fertilizer level have positively affected microbial population in different growing periods of the plant and different soil depths. Similarly, increasing B fertilizer levels have positively affected CO<sub>2</sub>-C production in different growing periods of the plant and different soil depths. The highest microbial population and CO<sub>2</sub>-C production was observed in 3 kg ha<sup>-1</sup> B level and in flowering period in 0 to 30 and 30 to 60 cm soil depths. High concentrations of boron in soils have caused toxicity and decreased soil microbial populations and soil respiration. Soil microbial population and soil CO<sub>2</sub>-C production were observed to be higher in superficial layer than in subsuperficial layer in all growing periods of the plant.

Increasing B fertilizer levels have shown positive effect on soil urease, phosphatase and dehydrogenase enzyme activities in different growing periods of the plant and different soil depths. Phosphatase and dehydrogenase enzyme activities of the soil have shown higher value in superficial layer than that of subsuperficial layer in all growing periods of the plant. Maximum urease enzyme activity has been observed in 6 kg ha<sup>-1</sup> B level and the highest phosphatase and dehydrogenase enzyme activities have been observed in 3 kg ha<sup>-1</sup> B level in harvest

period in both soil depth.

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