African Journal of Biotechnology Vol. 10(27), pp. 5311-5319, 15 June, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1376
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

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

Serdar Bilen[1,3]*, Murat Bilen[2] and Sougata Bardhan[3]

[1]University of Ataturk, Faculty of Agricultural, Department of Soil Science, Erzurum, Turkey.
[2]Eti Holding A., Research and Development Dept., Istanbul Road, 9th km, Güvercinlik, Ankara, Turkey.
[3]The Ohio State University, School of Environment and Natural Resources, 1680 Madison Avenue, Wooster, OH 44691, USA.

Accepted 14 March, 2011

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^{-1} 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_2-C production were observed at 3 kg ha^{-1} 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^{-1} B level and the highest phosphatase and dehydrogenase enzyme activities were observed in 3 kg ha^{-1} 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

*Corresponding author. E-mail: sbilen@atauni.edu.tr. Tel: +90 442 231 1646. Fax: +90 442 236 0958.

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_2 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, signification, 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 signifycantly 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^+ 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_2 species is weakened due to reduced levels of ascorbic acid, SH-compounds and H_2O_2 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_2-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^{-1}, 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^{-1} 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^{-1} as ammonium nitrate (33% N), 95 kg ha^{-1} P as diamonium phosphate (18% N, 46% P_2O_5), 150 kg ha^{-1} K as potassium sulfate (50% K_2O), and 0, 1, 3, 6 and 9 kg ha^{-1} Borax (11% B) (as $Na_2B_4O_7.10H_2O$), 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 $CaCO_3$ 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 (Hanlon 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 Aqumat 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 10^6 and 10^7 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_2 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_2 evolved over time was absorbed in the 0.5 M NaOH followed by precipitation as $BaCO_3$ by the addition of excess 1 M $BaCl_2$. 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:

BR rates (mg CO_2/kg soil) = (CO_2soil - CO_2air)/20 days

Urease enzyme (UE) activity was assayed by using urea solution and expressed as µg NH_4-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 l 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_2-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_2-C production

The quantitative analyses of soil bacteria, fungi, actionmycetes and CO_2-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^{-1}		18	6.65
$CaCO_3$ g kg–1		150	137.28
Total N g kg^{-1}		0.78	0.28
Available P mg kg^{-1}		9.3	3.28
Cation exchangeable capacity cmolc kg^{-1}		32.0	27.5
Exchangeable cations, cmolc kg^{-1}	Ca^{+2}	19.8	20.09
	Mg^{+2}	4.5	3.04
	K^{+1}	2.7	1.33
	Na^{+1}	0.15	0.10
Microelements, mg kg^{-1}	Fe^{+2}	3.36	1.32
	Mn^{+2}	1.74	1.17
	Zn^{+2}	1.75	1.14
	Cu^{+2}	0.78	0.69
	B	0.16	0.08
Electric conductivity dS m^{-1}		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^{-1} 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^{-1} B application in 0 to 30 and 30 to 60 cm soil depths in sowing, flowering and harvest periods (34.67, $15.27x10^6$ CFU g^{-1} soil; 47.50, $24.15x10^6$ CFU g^{-1} soil; 37.60, $21.05x10^6$ CFU g^{-1} soil, respectively). The number of bacteria decreased at B levels more than 3 kg ha^{-1} 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, $21.11x10^6$ CFU g^{-1} soil) (Table 2).

According to Table 2, the highest number of fungi were also observed in 3 kg ha^{-1} B level and in all growing period of plant in both soil depths (46.91 and $65.21x10^4$ CFU g^{-1} soil; 72.64, $95.80x10^4$ CFU g^{-1} soil; 61.25, $79.70x10^4$ CFU g^{-1} soil, respectively). The highest average number of fungi was observed in flowering period in both soil depths (62.52, $83.15x10^6$ CFU g^{-1} soil).

Similar observations were also made for actinomycetes with the highest average number at 3 kg ha^{-1} B application in 0 to 30 and 30 to 60 cm soil depths in all growing periods (21.00, $35.79x10^3$ CFU g^{-1} soil; 31.03, $55.07x10^3$ CFU g^{-1} soil; 24.19, $43.36x10^3$ CFU g^{-1} 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, $46.73x10^6$ CFU g^{-1} 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_2-C production was observed in 3 kg ha^{-1}

Table 2. Effect of boron applications on soil microbial population and CO_2-C production.

Soil	B	Microbial population									CO_2-C production		
Depth (cm)	Rate (kg da^{-1})	Bacteria			Fungi			Actinomycetes			CO_2-C		
		SP	FP	HP	SP	FP	HP	SP	FP	HP	SP	FP	HP
0-30	0	25.70 ^{b}	37.75 ^{b}	29.61 ^{ab}	36.16 ^{b}	57.03 ^{b}	46.77 ^{b}	16.99 ^{b}	22.25 ^{b}	16.65 ^{b}	6.48 ^{b}	9.94 ^{ab}	7.78 ^{b}
	1	31.42 ^{ab}	42.00 ^{ab}	30.36 ^{ab}	42.75 ^{ab}	67.30 ^{ab}	53.26 ^{ab}	17.33 ^{ab}	25.19 ^{ab}	19.85 ^{ab}	7.79 ^{ab}	11.52 ^{ab}	8.40 ^{ab}
	3	34.67 ^{a}	47.50 ^{a}	37.60 ^{a}	46.91 ^{a}	72.64 ^{a}	61.25 ^{a}	21.00 ^{a}	31.03 ^{a}	24.19 ^{a}	8.57 ^{a}	12.67 ^{a}	10.20 ^{a}
	6	25.37 ^{ab}	47.00 ^{a}	24.80 ^{b}	40.48 ^{ab}	72.00 ^{a}	59.12 ^{a}	17.66 ^{ab}	26.95 ^{ab}	20.46 ^{ab}	7.33 ^{ab}	12.55 ^{a}	8.26 ^{ab}
	9	13.34 ^{b}	30.40 ^{c}	16.90 ^{c}	25.44 ^{c}	43.63 ^{c}	35.68 ^{c}	11.66 ^{c}	14.71 ^{c}	11.43 ^{c}	4.96 ^{c}	7.70 ^{b}	4.94 ^{c}
Ave.		26.10 ^{b}	40.93 ^{a}	27.85 ^{b}	38.35 ^{c}	62.52 ^{a}	51.22 ^{b}	16.93 ^{b}	24.03 ^{a}	18.52 ^{b}	7.03 ^{b}	10.88 ^{a}	7.92 ^{b}
30-60	0	13.20 ^{b}	19.27 ^{b}	15.03 ^{ab}	52.10 ^{b}	76.29 ^{b}	59.67 ^{b}	25.93 ^{b}	42.39 ^{b}	33.19 ^{b}	2.71 ^{b}	4.15 ^{b}	3.42 ^{b}
	1	14.36 ^{a}	23.56 ^{a}	16.81 ^{ab}	60.14 ^{ab}	89.12 ^{ab}	63.98 ^{ab}	32.09 ^{a}	49.65 ^{ab}	36.81 ^{ab}	3.12 ^{a}	5.05 ^{a}	4.02 ^{ab}
	3	15.27 ^{a}	24.15 ^{a}	21.05 ^{a}	65.21 ^{a}	95.80 ^{a}	79.70 ^{a}	35.79 ^{a}	55.07 ^{a}	43.36 ^{a}	3.67 ^{a}	5.60 ^{a}	5.75 ^{a}
	6	14.00 ^{a}	24.00 ^{a}	19.79 ^{a}	57.37 ^{ab}	95.00 ^{a}	64.38 ^{ab}	29.93 ^{ab}	54.50 ^{a}	38.03 ^{ab}	3.23 ^{a}	5.21 ^{a}	3.92 ^{ab}
	9	8.34 ^{c}	14.57 ^{c}	11.23 ^{b}	39.02 ^{c}	59.54 ^{c}	39.36 ^{c}	18.89 ^{c}	32.02 ^{c}	21.29 ^{c}	2.45 ^{b}	3.29 ^{b}	2.74 ^{c}
Ave.		13.03 ^{c}	21.11 ^{a}	16.78 ^{b}	54.77 ^{c}	83.15 ^{a}	61.42 ^{b}	28.52 ^{c}	46.73 ^{a}	34.54 ^{b}	3.04 ^{c}	4.66 ^{a}	3.97 ^{b}

SP, Sowing period; FP, flowering period; HP, harvest period. Bacteria 10^{-6} CFU g^{-1} soil; Fungi 10^{-4} CFU g^{-1} soil; Actinomycetes 10^{-3} CFU g^{-1} soil, CO_2-C mg C m^{-2} h^{-1}.

B application level in both soil depths and for all growing periods (8.57, 3.67x10^{3} CFU g^{-1} soil; 12.67, 5.60x10^{3} CFU g^{-1} soil; 10.20, 5.75x10^{3} CFU g^{-1} soil, respectively). Average CO_2-C production increased with B application up to 3 kg ha^{-1} level but decreased at levels higher than 3 kg ha^{-1} B in all growing periods. The highest average CO_2-C production was observed in flowering period for both soil depths (10.88, 4.66x10^{6} CFU g^{-1} soil) with higherCO_2-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^{-1} B application level at sowing period in 0 to 30 cm upper layer (36.12 µg NH_4^+-N g^{-1} soil 2h^{-1}) and 0 kg ha^{-1} (control) B application level at sowing period in 30 to 60 cm lower layer (19.27 µg NH_4^+-N g^{-1} soil 2h^{-1}).

The highest urease activity was observed in 6 kg ha^{-1} boron application at harvest period in both soil depths (138.52 µg NH_4^+-N g^{-1} soil 2h^{-1}, r^2= 0.80, $p < 0.01$; 85.28 µg NH_4^+-N g^{-1} soil 2h^{-1}, r^2= 0.89, $p < 0.01$, respectively). The urease enzyme activity increased up to 6 kg ha^{-1} 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 subsuferficial layer (Figure 1).

The highest phosphatase enzyme activity was observed in 3 kg ha^{-1} boron application and harvest period in both soil depths (76.0 µg pNP g^{-1} soil h^{-1}, r^2= 0.99, $p < 0.01$; 41.96 µg pNP g^{-1} soil h^{-1}, r^2= 0.99, $p < 0.01$, respectively). The phosphatase enzyme activity increased up to 3 kg ha^{-1} 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

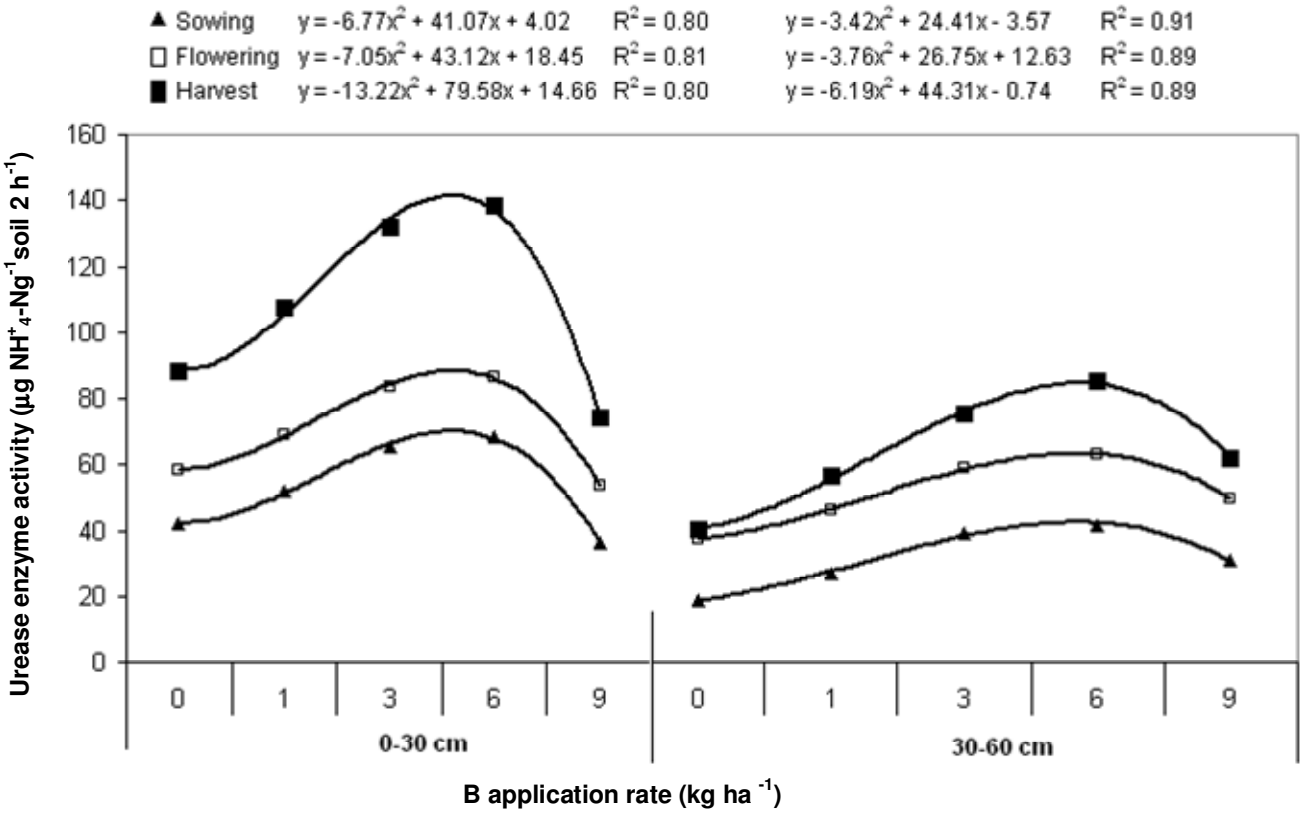

Figure 1. The effects of B application on urease enzyme activities of the soil in different soil depths.

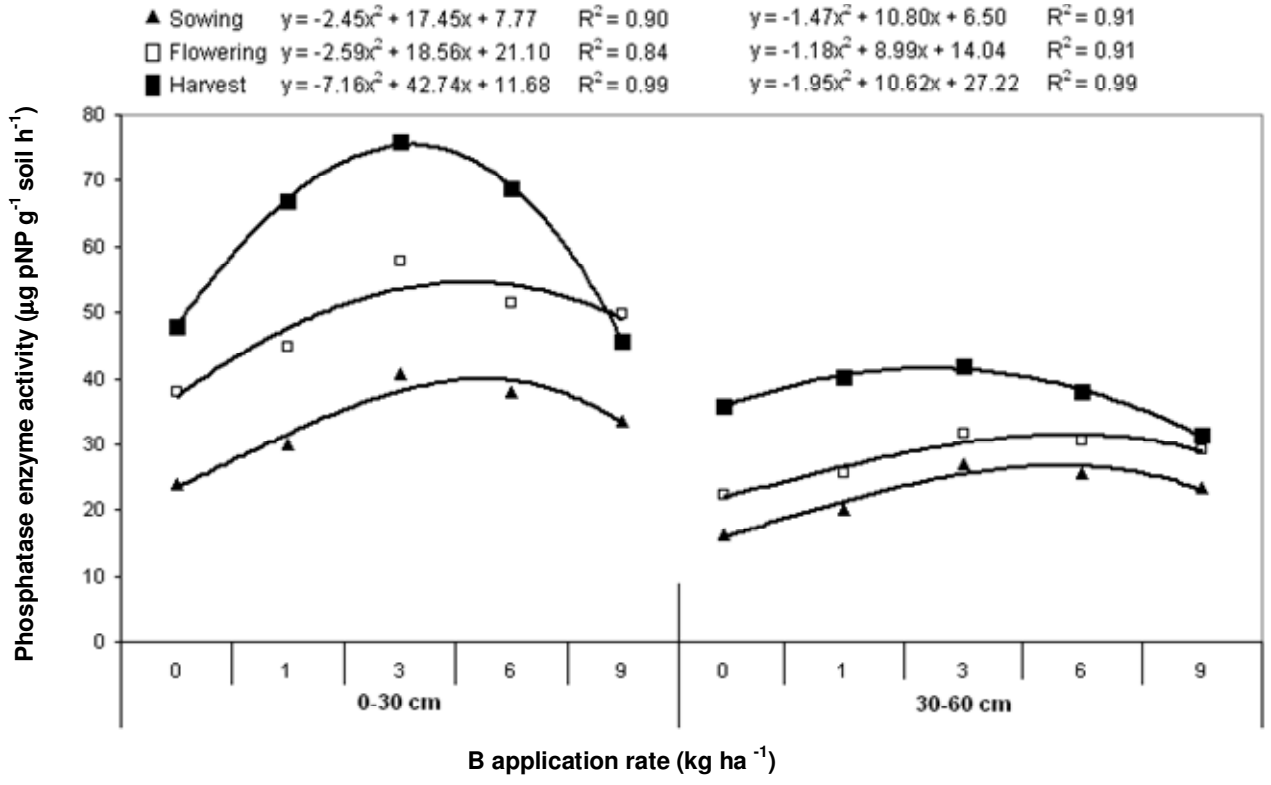

Figure 2. The effects of B application on phosphatase enzyme activities of the soil in different soil depths.

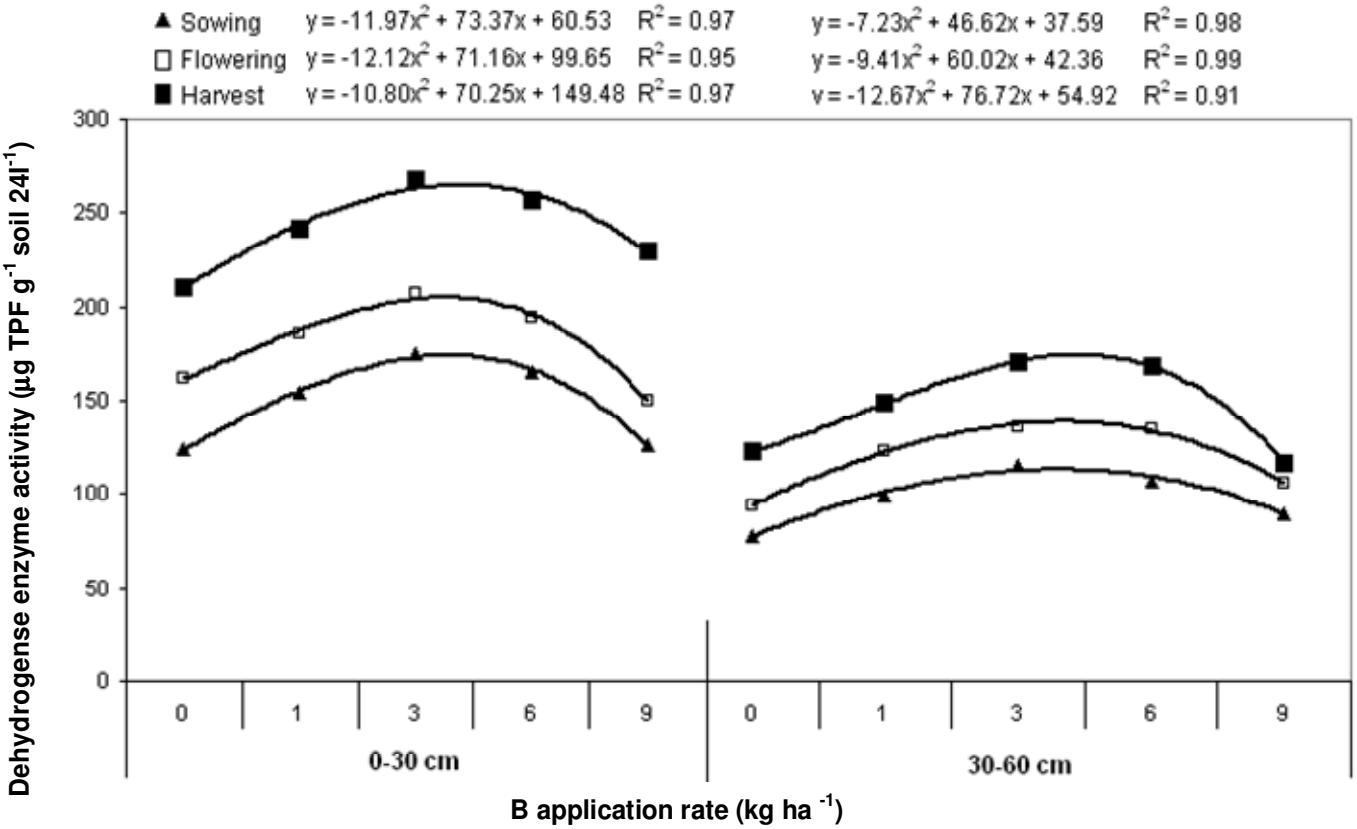

Figure 3. The effects of B application on dehydrogenase enzyme activities of the soil in different soil depths.

correlation with B application levels in different growing periods and in different soil depths. The highest dehydrogenase enzyme activity was observed at 3 kg ha^{-1} B application level and at harvest period in both soil depths (267.31 µg TPF g^{-1} soil 24 h^{-1}, r^2= 0.97, $p < 0.01$; 170.90 µg TPF g^{-1} soil 24 h^{-1}, r^2= 0.99, $p < 0.01$, respectively). The lowest was observed in 0 kg ha^{-1} (control) boron application and at sowing period in 0 to 30 cm (124.23 µg TPF g^{-1} soil 24 h^{-1}) and 30 to 60 cm (77.83 µg TPF g^{-1} soil 24 h^{-1}) soil depths. The dehydrogenase enzyme activity followed similar trend as alkaline phosphatase, increased up to 3 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). According 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 kg ha^{-1} B decreased soil microbial populations and CO_2-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_2-C production. Where microbial activity and CO_2-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_2-C production in different growing periods of the plant and different soil depths. The highest microbial population and CO_2-C production was observed in 3 kg ha^{-1} 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_2-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^{-1} B level and the highest phosphatase and dehydrogenase enzyme activeties have been observed in 3 kg ha^{-1} B level in harvest period in both soil depth.

REFERENCES

Acosta-Martinez V, Cruz L, Sotomayor-Ramirez D, Perez-Alegria L (2007). Enzyme activities as affected by soil properties and land use in a tropical watershed. Appl. Soil Ecol. 35(1): 35-45.

Ahmad W, Niaz A, Kanwal S, Rahmatullah MK, Rasheed MK (2009). Role of Boron in Plant Growth: A Review. J. Agric. Res. 47(3).

Alvarez-Tinaut MC (1990). Correlations between boron and other micronutrients. Behavior, Function and Significance of Boron in Agriculture. Report on an Intl. Workshop at St. John's College, Oxford, England. Published by Borax Consolidated Ltd, London. SW 1P 1H T.

Anantawiroon P, Subedi KD, Rerkasem B (1997). Screening wheat for boron efficiency, In: Bell RW and Rerkasem B (eds.). Boron in soil and plants. Proceedings. Kluwer Academic Publishers, Dordrecht, The Netherlands. pp. 101-104.

Angin I, Turan M, Ketterings QM, Cakıcı A (2008). Humic acid addition enhances B and Pb phytoextraction by Vetiver grass. Water Air Soil Pollut. 188: 335-343.

Anonymous (1995). Effective Boron Management. Though used in micro amounts, this vital nutrient plays a crucial role in a plant's health and development. FFF Review. Fluid J. http://www.fluidfertilizer.com/PastArt/pdf/10P20-23.pdf

Anonymous (2007). Micronutrients. Soil nutrient management for Maui County. College of Tropical Agriculture and Human Resources. University of Hawai'i. http://www.ctahr.hawaii.edu/mauisoil/c_nutrients04.aspx

Anonymous (2008). Total Soil Management. TSM Services, Inc. http://www.tsmser.com/TSM/mission/showmeexcess.htm

Asad A, Blamey FPC, Edwards DG (2002). Dry matter production and boron concentrations of vegetative and reproductive tissues of canola and sunflower plants grown in nutrient solution. Plant Soil, 243: 243-252.

Baradwaj KKR, Datt N (1995). Effects of legume green manuring on nitrogen mineralization and some microbiological properties in an acid rice soil. Biol. Fertil. Soils, 19: 19-21.

Berger KC, Pratt PJ (1963). Advances in secondary and micro-nutrient fertilization. In: McVickar MH, Bridger GL, Nelson LB (ed.). Fertilizer technology and use. SSSA, Madison, WI. pp. 281-340.Booij R (2000). Effects of nitrogen on yield components of Brussels sprouts (Brassica oleracea L. var. gemmifera DC). Eur. J. Horticult. Sci. 65: 30-34.

Bremner JM (1996). Nitrogen-Tolal. Methods of Soil Analysis, Part 3. Bartels JM, Bigham JM (Eds), ASA-SSSA, Madison, WI, pp. 1085-1123.

Brown PH, Shelp BJ (1997). Boron mobility in plants. Plant Soil. 193: 85-102.

Cakmak I, Romheld V (1997). Boron deficiency induced impairments of cellular functions in plants. Plant Soil, 193: 71-84.

Cakmak I (1994). Activity of ascorbate-dependent H_2O_2-scavenging enzymes and leaf chlorosis are enhanced in magnesium- and potassium-deficient leaves, but not in phosphorus-deficient leaves. J. Exp. Bot. 45: 1259-1266.

Canbolat MY, Bilen S, Cakmakci R, Sahin F, Aydin A (2006). Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. Biol. Fertil. Soils, 42(4): 350-357.

Carlos B (2000). Effects of Boron on Plants. Nevada's Horticulture Connection, University of Nevada Cooperative Extension. 1(1).

Ceyhan E, Onder M, Harmankaya M, Hamurcu M, Gezgin S (2007). Response of chickpea cultivars to application of boron in boron-deficient calcareous soils. Commun. Soil Sci. Plant Anal. 38: 2381-2399.

Cynathia SA (2003). Microbiological methods**.** 5th edition, Butlerworth publications, London.

Dick WA, Juma NG, Tabatabai MA (1983). Effects of soils on acid phosphatase and inorganic pyrophosphatase of corn roots, Soil Sci. 136: 19-25.

Dick WA, Tabatabai MA (1993). Significance and potential uses of soil enzymes. In: Metting FB (Ed.). Soil Microbial Ecol.: Application in Agricul. And Environmental Management. Marcel Dekker, New York. pp. 95-125.
FAO (1990). Micronutrient assesment at the levels an international study. FAO. Soil Bulletin, 63, Rome.
Fleming GA (1980). Essential micronutrients. I: Boron and molybdenum. In: Davis BE (ed.). Applied soil trace elements. John Wiley and Sons, New York. pp. 155-197.
Frankerberger WT, Dick WA (1983) Relationships between enzyme, activities and microbial growth and activity indices in soil. Soil Sci. Soc. Am. J. 47: 945-951.
Gee GW, Bauder JW (1986). Particle-size analysis. In: Klute A (Ed.). Methods of soil analysis. Part 1. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI. pp. 383-411.
Gezgin S, Hamurcu M (2006). The importance of the nutrient elements interaction and the interactions between boron with the other nutrients elements in plant nutrition. Selcuk University, J. Agricult. pp. 24-31.
Gezgin S, Dursun N, Hamurcu M, Ayasli Y (1999). Konya ovasında sekerpancarı bitkisinin beslenme sorunlarının toprak ve bitki analizleri ile belirlenmesi. Konya Pancar Ekicileri Kooperatifi Egitim ve Saglık Vakfi Yayınlari.
Goldberg S (1997). Reactions of boron with soils. Plant Soil, 193: 35-48.
Gupta UC (1971). Boron and molybdenum nutrition of wheat, barley and oats grown in Prince Edward Island soils. Can. J. Soil Sci. 51: 415-422.
Gupta UC (1993). Factors affecting boron uptake by plants, In: Gupta, U.C. (Ed.). Boron and its role in crop production. CRC Press Inc., Boca Raton, FL. pp. 87-104.
Haas E (1992). The Function of Soil Minerals and Trace Elements in Soil, Plant, Animal, and Human Nutrition and Health. Staying Healthy with Nutrition. Celestial Arts http://www.soilminerals.com/information.htm
Handershot WH, Lalande H, Duquette M (1993). Soil Reaction and Exchangeable Acidity. Soil Sampling and Methods of Analysis. Martin R. Carter (Ed.). Canadian Society of Soil Sci. Lewis Publishers. Boca Raton, Florida, USA. pp. 141-147.
Hanlon EA, DeVore JM (1989). IFAS extension soil testing laboratory chemical procedures and training manual. In: FL Coop. Ext. Serv., Circ. 812, Institute Food Agric. Sci. Univ. FL, Gainesville, FL.
Hu H, Brown PH (1994). Localisation of boron in the cell wall and its association with pectin. Evidence of a structural role of boron in the cell wall. Plant Physiol. 105: 681-689.
Islam KR, Weil RR (2000). Land use effect on soil quality in tropical forest ecosystem of Bangladesh. Agric. Ecosyst. Environ. 79: 9-16.
Khan B (2009). Environmental Impact and Health Effects of Boron. http://www.articlesbase.com/college-and-university-articles/environmental-impact-and-health-effects-of-boron-569905.html
Knudsen D, Beegle D (1988). Recommended phosphorous tests. In: W.C. Dahnke, Editor, Recommended chemical soil test procedures for the north central regionNorth Central Region Publication No. 221, pp. 12-15.
Kolesnikov SI, Popovich AA, Kazeev KSh, Val'kov VF (2008). The Influence of Fluorine, Boron, Selenium, and Arsenic Pollution on the Biological Properties of Ordinary Chernozems. Eurasian Soil Sci. 41(4): 400-404.
Leoppert RH, Suarez DL (1996). Carbonate and Gypsum. Methods of Soil Analysis, Part 3. Bartels JM, Bigham JM (Eds). ASA-SSSA, Madison, WI, pp. 437-475.
Lindsay WL, Norvell WA (1978). Development of a DTPA test for zinc, iron, manganese, and copper. Soil Sci. Soc. Am. J. 42: 421-428.
Loomis WD, Durst RW (1992). Chemistry and biology of boron. Bio Factors. 4: 229-239
Madigon MT, Martinko JM (2006). Biology of Microorganisms.11th Edition. Upper Saddle River, NJ; Pearson Prentice Hall.
Manchanda HR, Yadav OP (1978). Effect of boron application on barley in a sierozem sandy soil. Short Communication. Plant Soil, 50: 711-714.
Marschner H (1995). Mineral Nutrition of Higher Plants. Aca. Press, San Diego, USA, pp. 379-396.
Nelson DW, Sommers LE (1996). Total Carbon, Organic Carbon, and Organic Matter. Methods of Soil Analysis, Part 3. Bartels JM, Bigham JM. (Eds). ASA-SSSA, Madison, WI, pp. 961-1011.
Oyinlola EY (2007). Effect of boron fertilizer on yield and oil content of three sunflower cultivars in the Nigerian Savanna. J. Agron. 6: 421-426.
Perucci P (1992). Enzyme activity and microbial biomass in a field soil amended with municipal refuse. Biol. Fertil. Soils, 14: 54-60.
Pollard AS, Parr AJ, Loughman BC (1977). Boron in relation to membrane functions in higher plants. J. Exp. Bot. 28: 831-841.
Rhoades J (1996). Salinity: electrical conductivity and total dissolved solids. In: Methods of Soil Analysis: Part 3-Chemical Methods, Soil Sci. Soc. Am. Madison, WI, pp. 417-435.
Shelp BJ (1993). Physiology and biochemistry of boron in plants. In: Boron and its Role in Crop Protection. Gupta UC (Ed.). CRC Press, Boca Raton, FL, USA, pp. 53-85.
Shorrocks VM (1997). The occurrence and correction of boron deficiency. Plant Soil, 193: 121-148.
Simmons J (1998). Balancing soil nutrition. TurfNet Monthly. EarthWorks News and Events. http://www.soilfirst.com/tnm_02_1998.htm
Soil Survey Staff (1999). Soil Taxonomy a Basic System of Soil Classification for Making and Interpreting Soil Surveys 2nd ed. US Dept. of Agric. Soil Conservation Service Washington.
Soylu S, Topal A, Sade B, Akgun A, Gezgin S, Babaoglu M (2004). Yield and yield attributes as affected by boron application in boron deficient calcareous soils. An evaluation of major Turkish genotypes for boron efficiency. J. Plant Nutr. 27: 1077-1106.
Sumner ME, Miller PE (1996). Cation Exchange Capasity and Exchange Coefficients. Methods of Soil Analysis, Part 3. Bartels JM, Bigham JM (Eds), ASA-SSSA, Madison, WI, pp. 1201-1231.
Swaine BJ (1955). The trace elements content of soils. Common-Wealth Bureau of Soil Sci. (GB), Tech. Common 48.
Tabatabai MA (1994). Soil Enzymes. In: Weaver RW, Angle JS and Bottomley PS, Editors, Methods of Soil Analysis: Microbiological and Biochemical Properties. Part 2. SSSA Book Ser. 5, SSSA, Madison, WI, pp. 775-833.
Tariq M, Mot, CJB (2007). Effect of Boron on the Behavior of Nutrients in Soil-Plant Systems-A Review. Asian J. Plant Sci. 6(1): 195-202.
Turan M, Sevimli F (2005). Influence of different nitrogen sources and levels on ion content of cabbage (*Brassica oleracea* var. *capitate*). N. Zealand J. Crop Hort. Sci. 33: 241-249.
Wilcox LV (1960). Boron injury to plants. USDA. Bull. 211: 1-6.
Wolf B (1974). Improvements in the azomethine-H method for the determining of boron. Commun. Soil Sci. Plant Anal. 5: 39-44.