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ASSESSMENT OF SOIL BORON AVAILABILITY IN APPLE ORCHARDS USING DIFFERENT EXTRACTANTS

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ABSTRACT

Soil analysis for boron is vital as the range between its deficiency and toxicity is quite narrow and its application slightly above the optimum level may prove toxic to plants. In present study, the extractability of various extractants was assessed in relation to the physicochemical properties of soils. Soil sampling was carried out in 30 orchards in district Kulgam, Kashmir. Four extractants widely used to determine plant-available boron from either acidic or alkaline soils were used. The hot-water-soluble boron (HWSB) extraction procedure was used as a benchmark to observe relative variation in available boron in alternate extractants like calcium chloride-mannitol ($CaCl_2$-mannitol), ammonium acetate and diethylene-triaminepenta-acetic acid-sorbitol (DTPA-sorbitol). A significant correlation was found for available B among all the extractants tested, the strongest correlation (r = 0.975 at $P \leq 0.01$) was observed between HWSB and DTPA-sorbitol and hence it may substitute the hot water extraction method for estimating plant-available B.**

Key words: Apple, ammonium acetate, $CaCl_2$-mannitol, correlation, DTPA sorbitol, hot water, regression

INTRODUCTION

Boron (B) deficiency in crops has been reported worldwide (Shorrocks, 1997) and the crops facing shortage of B include apple, pear, beet root, mustard, celery, cabbage and cauliflower (Marshner, 1995). Leaching of boron out of the solum in coarse textured soils makes B unavailable to the plants. However, fine textured soils have adequate B quantity but some conditions like wet or dry and over liming of acid soils may become impediment to B uptake by plants and cause its deficiency (Ganie *et al*., 2013). Contrary to this B toxicity is patchy but may occur in some plants due to application of concentrated B sprays (Mortvedt and Woodruf, 1993). Despite the high requirement of B in apple trees (Hanson, 1993), they are highly sensitive to its excessive concentration nevertheless (Gupta *et al.*, 1985).

Boron deficiency leads to abortion of flower initials and poor set of fruit or seeds (Loomis and Durst, 1992). Boron deficient apple trees produce small, misshapen and corky fruits which are highly sensitive to cracking and develop a poor red colour (Ganie *et al*., 2013). Boron deficiency in apple trees is diagnosed by performing plant and/or soil analysis (Tsadilas and Kazai, 2005). However for B, the soil analysis is very important as the range between its deficiency and toxicity is pretty narrow (Reisenauer *et al*., 1973), thereby may prove toxic to plants when applied slightly

above the optimum level. This highlights the necessity for careful assessment of available B in soils for judicious use of B fertilizers.

To estimate plant available B in soils and for predicting the response of crops to applied B, a number of extractants have been tested e.g. hot water (Berger and Truog, 1944), ammonium acetate (Cartwright *et al*., 1983), $CaCl_2$- mannitol (Aitken *et al*., 1987), DTPA sorbitol (Miller *et al*., 2000), mannitol (Tsadilas and Kazai, 2005), pressurized hot water (Shiffler *et al*., 2005), hot $CaCl_2$ and KH_2PO_4 (Sarkar *et al*., 2008), HCl and AB-DTPA (Niaz *et al*., 2011), etc. Among these extractants hot water is most commonly used. In this method high temperature leads to desorption of B from the soil matrix besides being efficient to extract B from the organic and soluble pools of soil (Offiah and Axley, 1993). However, the method is tedious and time consuming, yields coloured extracts and often results in errors linked with the boiling process. The suitability of different extractants for assessing the plant available B in soil is a function of nature of soil and crop type. For example, $CaCl_2$-mannitol is apparently efficient in alkali soils, while its appropriateness in acid soils leave question marks (Aitken *et al*., 1987). Similarly, dilute acids may be better extractants for evaluating the availability of B in strongly weathered acid than for juvenile soils (Lindsay and Cox, 1985). Finally, no single extractant is fit for all the crops even when they are grown on the same soil. Very little information is available on the appropriateness of extractant for evaluating the status of available B in apple growing areas of district Kulgam (Kashmir). The present study was aimed to study the suitability of various extractants in assessing soil B availability for apple cultivation.

MATERIALS AND METHODS

Study area

Soil and leaf samples from 30 apple orchards representing three physiographic zones *viz*., low (1601-1750 m amsl), mid (1751-1900 m amsl) and high (>1900 m amsl) altitude zones of district Kulgam in Kashmir valley were collected and analyzed in the year 2013. The details of sampling sites are shown in geo-referenced map (Fig. 1). The soils from high altitude zones belongs to Mollisols and Entisols, mid altitude zones to Alfisols while as low altitude zones to Inceptisols.

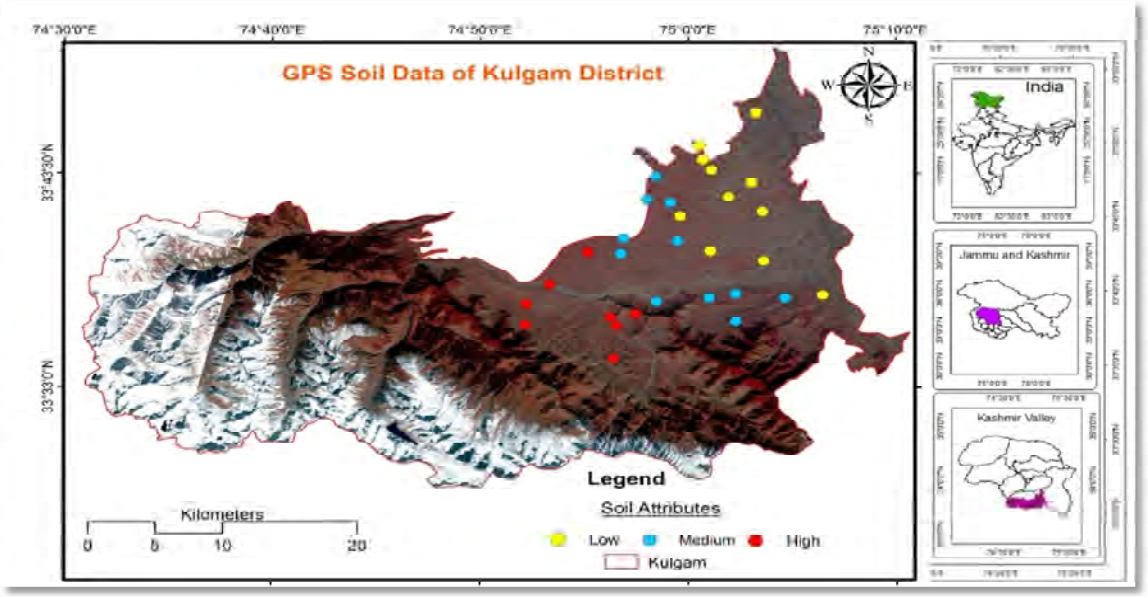

Fig. 1: Geo-referenced map showing sampling sites of the study area

Soil analysis

Thirty geo-referenced soil samples from apple orchards of district Kulgam (Kashmir) at three depths (0-15, 15-30 and 30-45 cm) were collected using global positioning system (GPS) after crop harvest in 2013. Collected soil samples were air dried in shade, ground with wooden mallet and passed through a 2 mm sieve. For determination of organic carbon, the samples were passed through a 0.2 mm sieve. Available B content was extracted by using various extractants. Boron was then estimated in extracts by using inductively coupled plasma optical emission spectrophotometer (ICP-OES) [Varian, Vista-MPX]. The extractants used included hot water (Berger and Truog, 1944), ammonium acetate (Cartwright *et al*., 1983), $CaCl_2$-mannitol (Cartwright *et al*., 1983) and DTPA-sorbitol (Miller *et al*., 2000). In hot water extraction method the soil: extractant ratio used was 25:50 and the samples were boiled for 5 min. and filtered. In rest extractants, the soil: extractant ratio used was 10:20 and the samples were then shaken for 60 min followed by centrifugation at 3500 rpm for 5 min. The extractants used other than hot water included ammonium acetate (1.0 M NH_4OAc; pH 7.0), $CaCl_2$-mannitol (0.01M $CaCl_2$ + 0.05 mannitol) and DTPA-sorbitol (0.2 M DTPA + 0.2 M sorbitol). Soil samples were analyzed for pH (1:2.5 soil: water), organic carbon (Walkley and Black, 1934), calcium carbonate (Puri, 1930), cation exchange capacity (Rhoades, 1982) and clay (Piper, 1966).

Leaf analysis

Composite leaf samples were collected from 30 apple orchards from 15 July to 15 August, 2013. In each orchard the samples were taken from randomly selected 25 trees, eight leaves from one tree from all the four directions. Moreover, leaves were sampled from the middle portion of current season shoot growth. Collected leaf samples were cleaned with tap water, dilute HCl (5 mL L^{-1} water) and distilled water. The cleaned samples were finally subject to oven drying at 65°C till the material exhibited constant weight. The dried material was ground to powder in Wiley grinding mill and ashed in muffle furnace at 550°C. The digested material was dissolved in 20% HCl, filtered and B concentration was estimated in the extracts by using ICP-OES.

Data analysis

Geographical information system (Version Arc GIS 10.2) was used for mapping the sampling locations of study area. Data regarding soil properties and B extracted by extractants was subjected to descriptive statistics to work out their mean, range and standard error. Besides, relationship between soil extractable B and leaf B content in apple orchards was worked out by correlation and regression analysis as carried out by Sarkar *et al*. (2008). However, influence of soil properties on extraction of B by various extractants was estimated by multiple regression (backward method).

RESULTS AND DISCUSSION

Soil physico-chemical properties

The mean values of pH, organic carbon, CEC, $CaCO_3$ and clay in surface soils of the study area were 6.11±0.06, 1.46±0.09 $cmol_{p+}$ kg^{-1}, 12.84± 0.53%, 0.91±0.13% and 27.46±0.75%, respectively. These observations are in line with Najar *et al*. (2002). The foliar B in collected samples ranged from 28.36 to 99.32 mg kg^{-1} with mean value of 57.14±3.8 mg kg^{-1}.

Boron extraction by hot water

The mean value of hot water soluble B in 0-15, 15-30 and 30-45 cm soil depth was 2.37, 2.27 and 2.23 mg kg^{-1}, respectively (Table 1). Results of similar magnitude were obtained by Goldberg *et al.* (2002); however, hot water soluble B extracted by Sarkar *et al.* (2008) and Niaz *et al*. (2011) was very low in magnitude perhaps due to the low B status of those soils. Currently, the standard plant

Table 1: Boron (mg kg^{-1}) content extracted by various extractants in apple orchard soils of district Kulgam (Kashmir)

Soil depth (cm)		HWS-boron	$CaCl_2$-mannitol	Ammonium acetate	DTPA-sorbitol
0-15	Range	0.88-4.67	0.18-1.78	1.14-5.95	0.74-3.91
	Mean	2.37±0.23	0.74±0.08	3.26±0.22	1.96±0.18
15-30	Range	0.86-4.56	0.20-1.71	1.14-5.96	0.72-3.85
	Mean	2.27±0.21	0.70±0.05	3.33±0.23	1.93±0.19
30-45	Range	0.78-4.66	0.15-1.70	1.01-5.76	0.67-3.85
	Mean	2.23±0.22	0.69±0.11	3.17±0.18	1.93±0.21
0-45	Range	0.78-4.67	0.15-1.78	1.01-5.96	0.67-3.91
	Mean	2.29±0.13	0.71±0.04	3.25±0.13	1.94±0.10
	CV (%)	53.65	59.26	37.48	50.22

CV = Coefficient of variation

available soil B test is a hot water extraction method (Berger and Truog, 1944; Mahler *et al*., 1984) that measures water-soluble B. Hot water soluble B has been found to be a useful index of plant responses to B fertilization in deficient soils and also of the B content in plants (Cartwright *et al*., 1983). In general such relationships have been obtained in mineral soils having concentrations of hot water soluble B upto 3 mg kg^{-1}. Despite its capacity to estimate plant available B, several workers have noted that 5 min. hot water extraction procedure is not convenient method and hence suggested modifications (Gupta and Stewart, 1975). The use of 5 min extraction has become general even though the short extraction time presents difficulties in maintaining uniformity of treatment before all the soils in a batch can be separated from their respective extracts. Several researchers have studied the kinetics of hot water soluble B method and observed that the recommended procedure did not achieve equilibrium conditions for the B extracted as a function of time. Further, they recommended a longer extraction period (10 min) to reduce relative timing errors between batches and operators or the use of special equipment to give close control over temperature and timing. Many other workers noted that in some soils the colour extracted from organic matter added problems for spectrophotometric determinations made with azomethine-H (Aitken *et al*., 1987). This problem was solved by using activated charcoal; however, the use of activated charcoal as decolourizing agent often produces high blank analysis. This method has some more challenges that result in inconsistent or sporadic use by many soil testing laboratories. Some of these challenges include B contamination from glassware, limited number of samples run at one time and inconsistency in refluxing time and lack of repeatable results.

Boron extraction by $CaCl_2$-mannitol

To overcome the problem encountered in coloured extracts in case of hot water soluble B method, Parker and Gardner (1981) used 0.02 M $CaCl_2$. Besides reducing colour in extract, it was noted that the colour developed with azomethine-H was stable for 4 h and the spectrophotometric determinations made with this reagent gave comparable results to the analysis made by ICP-OES. Hingston (1964) used 0.01 M $CaCl_2$ containing 0.01 M mannitol for desorption of B from clays. The mean value of B extracted by $CaCl_2$-mannitol in 0-15, 15-30 and 30-45 cm soil depth was 0.74, 0.70 and 0.69 mg kg^{-1}, respectively (Table 1). Results of similar magnitude were obtained by Sarkar *et al*. (2008) and Niaz *et al*. (2011); however, B extracted by this extractant was more than that of hot water soluble boron. Mannitol based extractants has been used for soil B extraction because of its ability to complex B. In present study, hot water soluble B was very high as compared to mannitol based extractant which may be because of the fact that soils studied in our case were acidic to almost neutral in reaction. Chemical studies on the nature of mannitol B complex have shown that the ability of mannitol to complex B is dependent on pH, with the concentration of mannitol-B complex decreasing with decreasing pH (Aitken *et al*., 1987). Knoeck and Taylor

(1969) concluded that mannitol reacted only with borate ion [$B(OH)^{4-}$] and not with boric acid to produce a complex. Since hydrolysis of boric acid to borate ion has a pK value of 9, therefore there will be very little or no borate ion in acidic conditions . Thus an unbuffered mannitol solution would not be expected to extract more B than water extract from acidic soils. Mannitol has been used as a B extractant in calcareous and alkaline soils (Rhoades *et al*., 1970).

Boron extraction by ammonium acetate

Ammonium acetate was also used as an alternative for hot water for extraction of plant available boron. Gupta and Stewart (1975) found that B extracted by ammonium acetate was as good as hot water to predict plant yield and the concentration and uptake of B in plants. In present study the highest B content was extracted with ammonium acetate (Table 3). The mean value of B extracted by ammonium acetate in 0-15, 15-30 and 30-45 cm soil depth was 3.26, 3.33 and 3.17 mg kg^{-1}, respectively. Results of similar magnitude were obtained by Sarkar *et al.* (2008) and Niaz *et al*. (2011); however, B extracted by using this extractant was more than that of hot water soluble B (Cartwright *et al*., 1983). Besides this Aitken *et al*. (1987) observed that 1.0 M ammonium acetate extracted similar amounts of B to those extracted with tartaric acid which has ability to complex boric acid. However, there is very little evidence in literature indicating that ammonium acetate can complex with boric acid.

Boron extraction by DTPA-sorbitol

Based on comparable effectiveness and reduced price, Vaughan and Howe (1994) recommended the use of another chelator, sorbitol, rather than mannitol in extracting B from a group of soils having diverse chemical and physical characteristics. DTPA-sorbitol method is recommended by the North American Proficiency Testing Program for estimating the potential soil bioavailability of zinc, copper, manganese, iron and boron (Miller *et al*., 2000). The mean value of B extracted by DTPA-sorbitol in 0-15, 15-30 and 30-45 cm soil depth was 1.96, 1.93 and 1.93 mg kg^{-1}, respectively (Table 1). These findings are in agreement with Goldberg *et al*. (2002) who reported soil B extractions as indicators of B content on field grown crops.

Relationship between soil extractable- and leaf-boron in apple orchards

At all the three soil depths leaf B content exhibited highest positive correlation with hot water soluble B (r = 0.98**) followed by DTPA-sorbitol extractable B (r = 0.95**) while lowest correlation was obtained with $CaCl_2$-mannitol extractable B (r = 0.52*) (Table 2). Linear regression curves were also plotted for B extracted by various extractants and leaf B content to determine the nature of their relationship as illustrated in Fig. 2. The relationship was described by the equations:

Table 2: Correlation coefficients (r) for soil extractable boron and leaf boron content

Soil depth (cm)	Extractants	$CaCl_2$-mannitol	Ammonium Acetate	DTPA-Sorbitol	Leaf B Content
0-15	HWS* boron	0.532*	0.917**	0.975**	0.979**
	$CaCl_2$ mannitol		0.537*	0.599*	0.526*
	Ammonium acetate			0.908**	0.902**
	DTPA-sorbitol				0.950**
15-30	HWS boron	0.609*	0.901**	0.980**	0.975**
	$CaCl_2$ mannitol		0.566*	0.607*	0.549*
	Ammonium acetate			0.906**	0.883**
	DTPA-sorbitol				0.950**
30-45	HWS boron	0.666*	0.892**	0.977**	0.973**
	$CaCl_2$ mannitol		0.575*	0.671*	0.655*
	Ammonium acetate			0.907**	0.860**
	DTPA-sorbitol				0.948**

HWS = * Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level

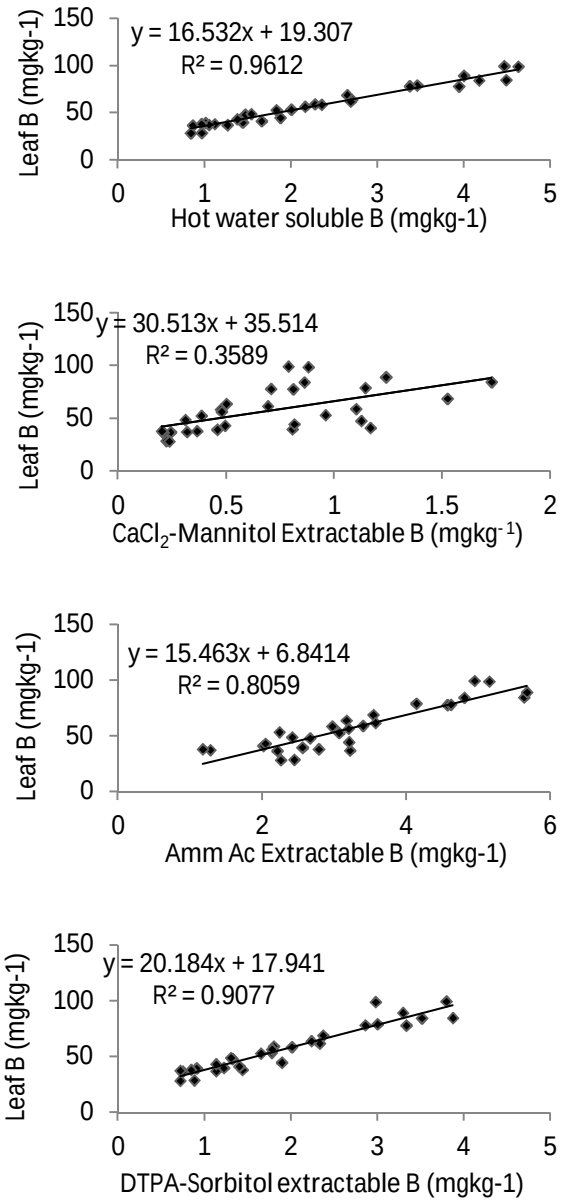

Fig. 2: Regression models of soil boron (average of three depths) extracted by various extractants and leaf boron content

Extractable B	Regression equation	(R^2)
Hot water	y = 16.532x + 19.31	0.9612
$CaCl_2$-mannitol	y = 30.513x + 35.51	0.3589
Ammonium acetate	y = 15.463x + 6.841	0.8059
DTPA-sorbitol	y = 20.184x + 17.94	0.9077

From the correlation and regression analysis it is explicit that correlation coefficient and coefficient of determination are very high between leaf B content and B extracted by hot water and DTPA-sorbitol. These results depict that for soil B extraction hot water and DTPA-sorbitol are best extractants. The results in Table 2 further unveiled the fact that at all the three soil depths highest positive correlation existed between hot water soluble B and DTPA-sorbitol (r = 0.98**). Further, linear regression curves plotted between hot water soluble B and B extracted by various extractants (Fig. 3) yielded following equations:

Extractable B	Regression equation	(R^2)
$CaCl_2$-mannitol	y = 1.7634x + 1.039	0.3637
Ammonium acetate	y = 0.908x - 0.6651	0.8128
DTPA-sorbitol	y = 1.2295x - 0.099	0.9538

High coefficient of determination and correlation coefficient between hot water soluble B and DTPA-sorbitol reveals that DTPA-sorbitol can replace hot water as an extractant for plant available B. These results corroborate with Shiffler *et al*. (2005) who suggested the use of DTPA-sorbitol in lieu of hot water because of the ease and relatively rapid extraction of soil available B. Contrarily, Niaz *et al*. (2011) found weak correlation between HWS-B and 0.25 M sorbitol-DTPA extractable B. The variations observed may be due to difference in soil reaction which was acidic to slightly neutral in present study and alkaline to calcareous in case of Niaz *et al*. (2011).

Influence of soil properties on extraction of boron by various extractants

Suitable extractant for B should be robust across a wide range of soil properties such as soil reaction, organic carbon, clay content, calcium carbonate content, cation exchange capacity, etc. Correlation study of soil properties revealed that hot water soluble B exhibited non-significant correlation with all the above soil properties at 0-15 cm depth (Table 3). However, very weak positive correlation was observed with organic carbon at 15-30 cm (r = 0.447*) and 30-45 cm (r = 0.382*). $CaCl_2$-mannitol exhibited non-significant correlation with the above soil properties at all the three depths. Similar was the case of ammonium acetate extractable B and DTPA-sorbitol B with only difference that ammonium acetate extractable B exhibited very weak positive correlation

Table 3: Correlation coefficients (r) for soil extractable boron and soil properties

Soil depth (cm)		HWS boron	$CaCl_2$-mannitol	Ammonium acetate	DTPA-sorbitol
0-15	pH	ns	ns	ns	ns
	Organic carbon	ns	ns	ns	ns
	CEC	ns	ns	ns	ns
	$CaCO_3$	ns	ns	ns	ns
	Clay	ns	ns	0.349*	0.377*
15-30	pH	ns	ns	ns	0.397*
	Organic carbon	0.447*	ns	ns	ns
	CEC	ns	ns	ns	ns
	$CaCO_3$	ns	ns	ns	ns
	Clay	ns	ns	ns	0.376*
30-45	pH	ns	ns	ns	ns
	Organic carbon	0.382*	ns	ns	0.424*
	CEC	ns	ns	ns	ns
	$CaCO_3$	ns	ns	ns	ns
	Clay	ns	ns	ns	ns

*Correlation is significant at the 0.05 level

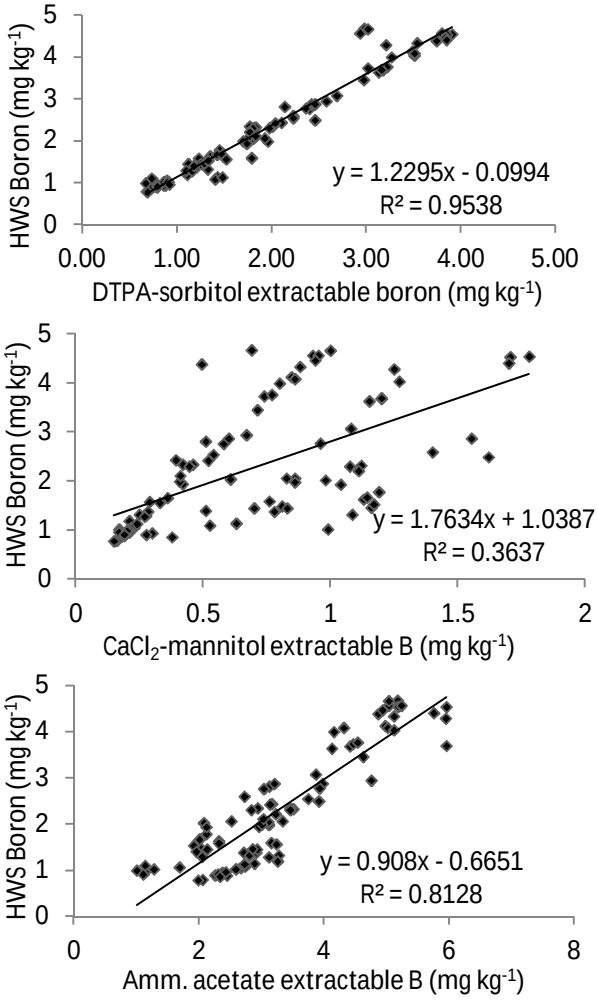

Fig. 3: Relation between HWSB, $CaCl_2$-mannitol, ammonium acetate extractable B and DTPA-sorbitol

with clay (r = 0.349*) at 0-15 cm depth and DTPA-sorbitol exhibited very weak positive correlation with clay (r = 0.377*) at 0-15 cm depth, pH (r = 0.397*) and clay (r = 0.376*) at 15-30 cm depth and organic carbon (r = 0.424*) at 30-45 cm depth.

Multiple regression models were computed using soil B extracted by various extractants and above soil properties as independent variables and leaf B content as dependent variable (Table 4). Best subsets were worked out for all extractants at all the three depths. The results further revealed that adjusted R^2 was highest in case of hot water soluble B (0.96), followed by DTPA sorbitol (0.90). While as least value for adjusted R^2 (0.34) was obtained in case of $CaCl_2$-mannitol. Further, the addition of soil properties improved adjusted R^2 significantly only for ammonium acetate and $CaCl_2$-mannitol extractants but not for hot water and DTPA-sorbitol. These results prove the superiority of hot water followed by DTPA-sorbitol over rest two B extractants. Similar findings were reported by Matsi *et al*. (2000) and Sarkar *et al*. (2008).

Conclusion: From the study it may be concluded that the extractants *viz*., hot water and DTPA-sorbitol are superior over other boron extractants for assessing the available B status in apple growing soils. Moreover, DTPA-sorbitol can be used as an alternative

Table 4: Best subsets determined by multiple regression (backward method) for boron content in apple leaves

Extractants	Soil depth (cm)	Best subsets	Regression model	Adjusted R^2
Hot water	0-15	Soil B + $CaCO_3$	21.287 +15.898 (soil B) –1.937 ($CaCO_3$)	0.96
	15-30	Soil B + OC + pH	46.585 +16.799 (soil B) –3.788 (pH) – 3.287(OC)	0.95
	30-45	Soil B + OC + pH + clay	33.067 +16.162 (soil B) –3.838 (pH)–3.386 (OC) + 0.561 (clay)	0.96
$CaCl_2$-mannitol	0-15	Soil B + clay	–7.928 + 26.841 (soil B) +1.646 (clay)	0.34
	15-30	Soil B + pH	110.703 + 25.320 (soil B) –11.501 (pH)	0.36
	30-45	Soil B + OC	24.393 + 28.774 (soil B) +12.918 (OC)	0.53
Ammonium acetate	0-15	Soil B + pH	36.327 + 15.214 (soil B) –4.872 (pH)	0.83
	15-30	Soil B + pH	56.768 + 13.850 (soil B) –7.386 (pH)	0.81
	30-45	Soil B + pH	86.186 + 13.128 (soil B) –11.308 (pH)	0.80
DTPA-sorbitol	0-15	Soil B	17.885 + 19.993 (soil B)	0.90
	15-30	Soil B	18.036 + 20.246 (soil B)	0.90
	30-45	Soil B + $CaCO_3$	22.628 + 19.803 (soil B) –4.130 ($CaCO_3$)	0.91

to hot water extraction method, which shows laboratory difficulties and is time-consuming.

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