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Full Research Article

Evaluation of Different Extractants and Profile Distribution of Boron in Guava Orchard in an Inceptisol of Bihar

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Abstract

The complex behavior of boron (B) in plant and soil systems makes it difficult to develop a suitable soil B extraction method that can determine the available nutrient status relative to plant needs under a wide range of soil conditions for various plant species. A suitable method for boron analysis must embrace the soil properties that determine the availability of Boron and acquiescent to routine laboratory analysis. On the basis of above premise three extractants, hot calcium chloride ($CaCl_2$), Potassium dihydrogen Phosphate (KH_2PO_4) and dilute Hydrochloric acid (HCl) solutions were evaluated against the soils of Guava orchard. Soil samples were collected from three Guava orchards blocks at three different depths (0-15, 15-30 and 30-45 cm) and analyzed. The order of extracted B by the three extractants were KH_2PO_4>hot $CaCl_2$>HCl. The amount of Boron extracted by each of the three extractants showed a gradual decrease with increase in depth. The amounts of B extracted by three extractants were also significantly correlated with each other; hot $CaCl_2 \leftrightarrow KH_2PO_4$ ($r=0.561^{**}$), $KH_2PO_4 \leftrightarrow HCl$, ($r=0.484^{**}$) and $HCl \leftrightarrow$ hot $CaCl_2$, ($r=0.642^{**}$). This shows dynamic equilibrium among the amount of B extracted by the extractants, indicating pools of extracted B in soils. The leaf samples from each tree were also collected and analyzed. B content of leaf tissue increased with increase in available B content in soil. Hot $CaCl_2$ extracted B is more correlated with leaf B content in comparison to other extractants in different soil layer.

1. Introduction

Guava (*Psidium guajava* L. Family Myrtaceae) has attained commercial importance in the tropics and subtropics because of its wide adaptability to varied soil and climatic conditions and as a prolific bearer (Fida et al., 2011). Bihar is the fourth largest Guava producing state in the country with the production of 0.24 mt from an area of 0.03 mha having productivity of 8.0 t ha^{-1}. Bihar is producing about 9.6% of total Guava in the country (NHM, State wise Horticulture Status, 2012). Yield reduction in the fruit orchards may be attributed to several factors. Among those the nutritional status of the soil may be a limiting factor.

Boron (B) is an essential trace element indispensable for the normal growth and development of plant. This micronutrient has a relatively narrow range between its phytotoxic and deficient limit in the soil. The deficiency of B is gaining importance in large areas especially coarse textured and calcareous soils with low organic matter content. In an average, 33% of Indian soils are B-deficient (Tiwari, 2006). Indian soils, particularly eastern and northeastern parts including Bihar, suffer from the B deficiency (Mondal et al., 1991; Dwivedi et al., 1993; Sarkar et al., 2006). Widespread B deficiency in soils is appearing even in fruit orchards in some areas as reported from different parts of World.

Leaf B content plays a major role in crop production. The increase in available B in soil increases the amount of leaf B content. The transpiration loss which was more from leaves and resulted in more movement of applied boron with water in the xylem to the leaves but due to phloem immobility of boron, there was more accumulation of boron in the leaves. Leaf boron content directly influenced the flower development, pollen tube growth, pollen viability, cell division and differentiation. Ultimately, it results into differentiation in the growth of fruit and development of pods in the crop (Padbhushan and Kumar, 2014). Upper surface (0−15 cm) of soil profile containing substantial amount of organic carbon may contain higher amounts of available boron. Knowledge of vertical distribution of boron in orchard soils is important because it indicates the depletion as well as accumulation pattern of B, if any, within the soil profile. Moreover, the roots of many crops especially fruit plants go beyond surface soil layers and draw their nutrient from the deeper layers. Guava is also not an exception regarding the proliferation of roots in the deeper layer of soil and research studies in this arena of fruit crops mainly orchard crops is currently lacking. In this study a modest initiative has been taken to visualize the depth wise distribution of Boron in the Guava orchards. Different extractants having diverse power of extraction and also from different pools of the soil, as mentioned in Table 1. were used to quantify B status in different depths. This design was formulated considering disparate soil properties with different depths.

Table 1: Summary of Soil B extraction methods used for B extraction in the experiment

Soil B extractant	Soil: Extractant	Method	References
0.02 M hot $CaCl_2$	1:2	Reflux 10 min. on hot plate	Parker and Gardner (1981)
0.5 M KH_2PO_4	1:2	Shake for 60 min.	Bloesch, Bell and Hughes (1987)
0.05 N HCl	1:2	Shake for 5 min.	(Ponnamperuma et al., 1981)

2. Materials and Methods

2.1. Soil and plant sampling

Three Guava (Var: Allahabad Safeda) orchardblocks of 5 years old in the agricultural farm of Bihar Agricultural University (24°14´N, 87°2´E and 12 m amsl), Sabour was selected for the study during 2015. The nutrient management of the orchard comprises 500:200:500 g NPK plant^{-1} along with FYM @ 20 kg plant^{-1} at the time of establishment. Block I consists of 169 trees whereas Block II and Block III consists of 64 and 56 trees respectively. Soil samples were collected randomly from each orchard block at 0–15, 15–30 and 30–45 cm from the outer periphery of the selected tree with the help of soil augerduring the month of December. After air drying soil samples were ground and passed through 2 mm sieve for laboratory analysis. At same time of soil sampling 3rd pair of recently matured leaves comprising a sample size of 25 was collected from each of the selected trees (Bhargava and Chadha, 1988). The leaf samples were processed for laboratory analysis.

2.2. Soil and leaf analysis

The air dried soil samples were analyzed for pH [in 1:2.5 soil-water suspension (Jackson, 1973)], organic carbon (OC) (Walkley and Black, 1934), clay (International pipette method), and amorphous Fe and Al oxides and Manganese (Mn) oxides [by extracting with 0.02 M ammonium oxalate, pH 3.0 (McKeague and Day, 1966)]. Available B was then extracted by three different extractants, hot $CaCl_2$, KH_2PO_4

Table 2: Important physical and chemical properties of the soils collected from different depths (0–15, 15–30 and 30–45 cm) of three Guava orchard blocks

Char-acter-istics	pH			Clay (g kg^{-1})			Organic Carbon (g kg^{-1})			Amorphous Fe (g kg^{-1})			Amorphous Al (g kg^{-1})			Mn-oxides (g kg^{-1})		
Soil depth (cm)	0–15	15–30	30–45	0–15	15–30	30–45	0–15	15–30	30–45	0–15	15–30	30–45	0–15	15–30	30–45	0–15	15–30	30–45
Block I																		
Range	6.10-7.40	6.10-7.61	6.12-7.65	120-140	140-170	150-210	9.6-12.8	4.1-12.3	2.10-7.90	3.15-8.40	1.83-7.64	2.31-6.61	2.15-5.23	3.46-5.56	1.12-4.78	0.56-1.16	0.52-0.97	0.72-1.16
Mean	6.98	7.20	7.30	132	155	176	10.21	8.30	4.42	4.44	4.97	2.82	2.32	3.12	2.95	0.78	0.71	0.82
Block II																		
Range	5.73-7.20	6.45-7.26	6.81-7.43	128-157	130-180	130-160	7.5-14.7	6.6-11.7	4.1-9.2	2.77-6.30	2.12-7.07	2.51-5.14	2.46-4.27	2.31-4.17	1.17-3.19	0.61-1.28	0.51-1.45	0.57-1.35
Mean	6.57	6.80	7.06	140	155	145	11.4	10.2	6.6	4.40	4.50	2.65	2.35	3.22	1.67	0.71	0.93	0.94
Block III																		
Range	5.79-7.09	6.12-7.10	6.53-7.20	140-165	140-280	120-240	4.8-8.4	4.5-9.4	3.5-8.8	3.02-7.40	2.43-6.64	2.56-6.61	1.46-4.17	1.13-3.27	1.27-3.42	0.14-2.13	0.61-2.10	0.43-1.54
Mean	6.57	6.77	7.08	150	208	200	6.2	6.6	5.4	5.12	4.51	4.58	2.71	2.22	2.24	1.16	1.27	0.93

and HCl, whose details are given in Table 1. Five (5.0 ml) of sample aliquot, 2.0 ml of ammonium acetate buffer (pH 5.5) and 2.0 ml of 0.02 M EDTA were added in a 20.0 ml B free test tube and vortexed. After adding 1.0 ml of 0.9% Azomethine-H solution (Wolf, 1971) the tube was again vortexed, allowed to stand for one hour at 20–25 °C, vortexed again and the readings were taken at 420 nm using HALO DB-20S, Australia UV-VIS double beam spectrophotometer.

The leaf samples were washed first in running tap water followed by dilute hydrochloric acid solution and finally with deionized water. The leaf materials were dried first in air and then in an oven at 70 °C. Samples were then ground with the help of a stainless steel mechanical grinder. Representative samples were dry ashed at 550 °C in a muffle furnace and then after cooling extracted with 0.36 N H_2SO_4 following the method as determined by Gains and Mitchell (1979) with only slight modification of time allowed for colour development, which was one and half an hour in lieu of just one hour used by the former. Extractable B was determined colorimetric ally by the azomethine-H method (Parker and Gardner, 1981).

2.3. Statistical analysis

Correlation was performed to evaluate the relationships among the various parameters. Statistical analysis was done by Microsoft Excel (Microsoft Corporation, USA) and SPSS window version 16.0 (SPSS Inc., Chicago, USA).

3. Results and Discussion

3.1. Physical and chemical properties of soil

Among the physico-chemical properties the attribute that directly govern or influence the B availability in the soil were taken into consideration like pH, clay content, organic carbon (OC), amorphous Fe, amorphous Al and Mn-oxides as portrayed in Table 1.

The characteristics of pH from all the three guava orchard blocks ranges between slightly acidic to alkaline in the three different soil depths (0–15, 15–30 and 30–45 cm). Soil pH of sub-surface horizons was found to have higher pH values than surface horizons in all the orchards. Leaching of bases along with the percolating water might caused increase in soil pH with increase in depth. Increase in soil pH with increase in depth indicates accumulation of bases.

A variation in clay content in different depths of the three blocks were observed. The clay content increased with the increase in depth irrespective of orchard blocks. The oxidisable organic carbon decreases with increase in soil depths in different blocks of orchard. The mean OC was more in surface layer than subsurface layer is due to addition of more organic matter by addition of leaf litter from the tree and more congenial environment for microbial activity resulting into more supplementation of OC.

The amorphous Fe, Al and oxides of Mn are the soil parameters plays a major role in the determining the nutrient bounding capacity especially B. The amount of these parameters in the soil depends upon the soil forming properties and weathered rock materials from which they are formed.

3.2. Soil bcontent using different extractants and B content in leaf tissue

The results (Table 3). show that the amount of B extracted by the three different extractants-hot $CaCl_2$, KH_2PO_4, and HCl-in surface-layer soil ranged from 0.38 to 0.82 , 0.16 to 1.09 and 0.12 to 0.77 mg kg^{-1} with mean values of 0.56, 0.57 and 0.40 mg kg^{-1}, respectively, in Block I. The ranges for Block II for these three extractants were 0.42 to 0.82, 0.50 to 1.33, and 0.10 to 0.57 mg kg^{-1} with mean values of 0.61, 0.85, and 0.40 mg kg^{-1}, and those for Block III were 0.63 to 1.23, 0.91 to 2.08, and 0.29 to 1.09 mg kg^{-1} with mean values of 0.87, 1.49, and 0.63 mg kg^{-1}, respectively. The amounts of B extracted by the extractant thus varied with soil properties in different blocks. The order of extractability was KH_2PO_4>hot $CaCl_2$>HCl irrespective of the different blocks. (Sarkar et al., 2008) reported the magnitude of efficacy of B extraction as follows hot $CaCl_2$>KH_2PO_4>Tartaric Acid for Inceptisols, Tartaric Acid> hot $CaCl_2$>PDP for Entisols, and KH_2PO_4>hot $CaCl_2$>Tartaric Acid for Alfisols and showed a decrease along soil depth. This might be due to an effective desorption of B from inorganic constituents such as the oxides and hydroxides of Fe and Al by the phosphate ion in the extractant, because phosphate has a higher bonding energy constant than that of borate (Bloesch et al., 1987). The amount of Hot $CaCl_2$ B is more than the HCl B. This might be due to hot $CaCl_2$ extract B even from organic, adsorbed, and soluble pools in soils in comparison to HCl (Sarkar et al., 2008).

The leaf boron content range in Block I is 15.4–42.1 mg kg^{-1} with mean value 22.6 mg kg^{-1}, in Block II 14.1–55.1 with mean value 28.9 and Block III 19.2–53.7 with mean value 35.9 mg kg^{-1}. The mean leaf B content and mean soil B content was maximum in Block III (Table 3). This shows that the leaf boron content of the leaf increased with increase in available boron content in the soil which was highest in Block III.

3.3. Depth wise distribution of soil B

Figure 1 represents the B extracted using the three extractants (Hot $CaCl_2$, KH_2PO_4 and HCl) had a gradual decline in amount with respect to soil depth. The maximum B content was observed in surface layer and minimum in subsurface layer in all the three blocks of guava orchard. The magnitude of decrease in B content in soil layer 15–30 cm with respect to 0–15 cm was 37.5, 19.3, 42.8; 42.6, 30.5, 40.0 and 37.9, 18.1, 49.2% for hot $CaCl_2$, KH_2PO_4, HCl in Block I, II and III, respectively. Similarly, in soil layer 30–45 cm with respect to 0–15 cm was 58.9, 52.6, 52.5; 73.8, 50.5, 57.5 and 75.8, 34.2, 68.2% respectively for above mentioned extractant and blocks of guava orchard

Table 3: Depth-wise distribution of extractable B of soils with three different extractants and B content in leaf tissue of three Guava orchard blocks

Extractants	Hot $CaCl_2$ B			KH_2PO_4 B			HCl B			B content in leaf tissue (mg kg^{-1} dry matter)
Soil depths (cm)	0-15	15-30	30-45	0-15	15-30	30-45	0-15	15-30	30-45	
Block I	(mg kg^{-1})									
1.	0.52	0.41	0.26	0.63	0.56	0.12	0.19	0.13	ND	16.4
2.	0.61	0.53	0.22	0.59	0.51	0.49	0.23	0.17	0.11	17.8
3.	0.57	0.45	0.19	0.31	0.36	0.23	0.32	0.27	0.14	17.4
4.	0.50	0.34	0.19	0.16	0.12	ND	0.21	0.16	ND	16.1
5.	0.61	0.33	0.21	0.63	0.59	0.51	0.51	0.49	0.31	17.2
6.	0.68	0.31	0.15	0.65	0.61	0.51	0.67	0.39	0.31	20.4
7.	0.82	0.67	0.46	0.65	0.43	0.37	0.55	0.40	0.10	23.6
8.	0.70	0.57	0.34	0.70	0.58	0.44	0.65	0.51	0.30	32.1
9.	0.42	0.23	ND	1.09	0.87	0.12	0.77	0.60	ND	30.4
10.	0.53	0.31	ND	0.39	0.32	0.11	0.12	0.10	ND	28.6
11.	0.71	0.42	0.18	0.54	0.43	0.31	0.31	0.19	0.13	34.4
12.	0.52	0.40	0.21	0.53	0.47	0.39	0.42	0.30	0.17	15.4
13.	0.64	0.20	0.32	0.63	0.51	0.22	0.56	0.32	0.11	26.2
14.	0.61	0.30	0.23	0.67	0.23	0.12	0.45	0.28	0.16	23.7
15.	0.60	0.55	0.41	0.70	0.41	0.37	0.58	0.41	0.30	33.8
16.	0.44	0.32	0.18	0.69	0.61	0.45	0.47	0.41	0.22	14.6
17.	0.72	0.49	0.26	0.34	0.21	0.17	0.51	0.31	0.19	42.1
18.	0.42	0.18	ND	0.69	0.55	0.32	0.31	0.20	0.11	18.6
19.	0.44	0.34	0.13	0.78	0.67	0.15	0.37	0.17	ND	19.1
20.	0.70	0.17	0.11	0.51	0.33	0.09	0.38	0.25	ND	23.3
21.	0.43	0.27	ND	0.46	0.41	0.10	0.23	0.16	ND	16.4
22.	0.38	0.10	ND	0.71	0.51	0.13	0.28	0.19	ND	17.5
23.	0.43	0.17	0.12	0.30	0.43	0.30	0.32	0.23	0.21	15.6
24.	0.41	0.17	0.19	0.60	0.49	0.27	0.29	0.09	ND	19.2
25.	0.56	0.45	0.18	0.31	0.26	0.18	0.38	0.22	ND	25.6
Mean	0.56	0.35	0.23	0.57	0.46	0.27	0.40	0.28	0.19	22.6
Block II										
1.	0.72	0.33	0.13	0.50	0.61	0.43	0.10	0.08	0.09	43.1
2.	0.53	0.41	0.16	0.50	0.24	0.17	0.57	0.34	0.21	21.6
3.	0.42	0.36	0.10	1.08	0.54	0.18	0.52	0.32	0.18	18.9
4.	0.89	0.53	0.34	0.83	0.34	0.37	0.51	0.19	0.11	37.6
5.	0.52	0.31	0.14	0.48	0.31	0.22	0.36	0.15	ND	21.1
6.	0.68	0.26	0.12	0.94	0.73	0.56	0.31	0.12	ND	18.5
7.	0.50	0.17	ND	1.02	1.21	0.87	0.44	0.41	0.32	21.8
8.	0.61	0.36	0.10	1.33	1.00	0.73	0.42	0.37	0.25	23.8
9.	0.47	0.29	0.11	1.07	0.71	0.57	0.37	0.28	0.13	31.3
10.	0.55	0.37	0.13	0.90	0.77	0.63	0.37	0.24	0.17	20.7
11.	0.67	0.40	0.19	1.15	0.61	0.41	0.28	0.24	0.15	14.1
12.	0.82	0.51	0.31	0.89	0.64	0.47	0.39	0.25	0.15	39.7

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13.	0.77	0.56	0.23	0.50	0.61	0.43	0.10	0.08	0.09	56.3
14.	0.45	0.21	0.11	0.50	0.24	0.17	0.57	0.34	0.21	22.2
15.	0.78	0.33	0.16	1.08	0.54	0.18	0.52	0.32	0.18	55.1
16.	0.42	0.22	0.08	0.83	0.34	0.37	0.51	0.19	0.11	17.3
Mean	0.61	0.35	0.16	0.85	0.59	0.42	0.40	0.24	0.17	28.9
Block III										
1.	0.99	0.62	0.31	1.84	0.85	0.67	0.97	0.40	0.31	37.1
2.	0.77	0.59	0.33	0.91	1.62	0.93	1.09	0.36	0.22	43.2
3.	0.63	0.23	0.12	1.41	1.11	0.94	0.33	0.26	0.11	25.5
4.	0.81	0.57	0.31	1.99	1.54	1.21	0.52	0.42	0.31	46.2
5.	0.72	0.66	0.21	1.46	1.64	1.34	0.76	0.26	0.15	21.0
6.	0.88	0.59	0.33	1.30	0.87	0.78	0.73	0.49	0.28	24.4
7.	0.70	0.42	0.17	1.22	1.12	0.91	0.54	0.22	0.10	19.2
8.	0.64	0.57	0.22	1.84	1.82	1.57	0.60	0.27	ND	23.1
9.	0.71	0.33	0.19	1.17	1.07	0.93	0.40	0.37	0.23	25.5
10.	0.89	0.51	0.24	1.02	0.92	0.81	0.68	0.21	0.16	34.5
11.	1.12	0.78	0.31	2.08	1.60	1.23	0.77	0.33	0.12	53.7
12.	0.69	0.49	0.27	1.24	0.92	0.69	0.29	0.25	0.18	31.6
13.	1.23	0.82	0.47	1.86	1.33	0.95	0.74	0.49	0.21	51.2
14.	1.22	0.55	0.39	1.73	1.40	1.21	0.58	0.23	0.14	49.4
15.	1.13	0.44	0.29	1.32	0.54	0.47	0.49	0.27	0.22	52.3
Mean	0.87	0.54	0.28	1.49	1.22	0.98	0.63	0.32	0.20	35.9

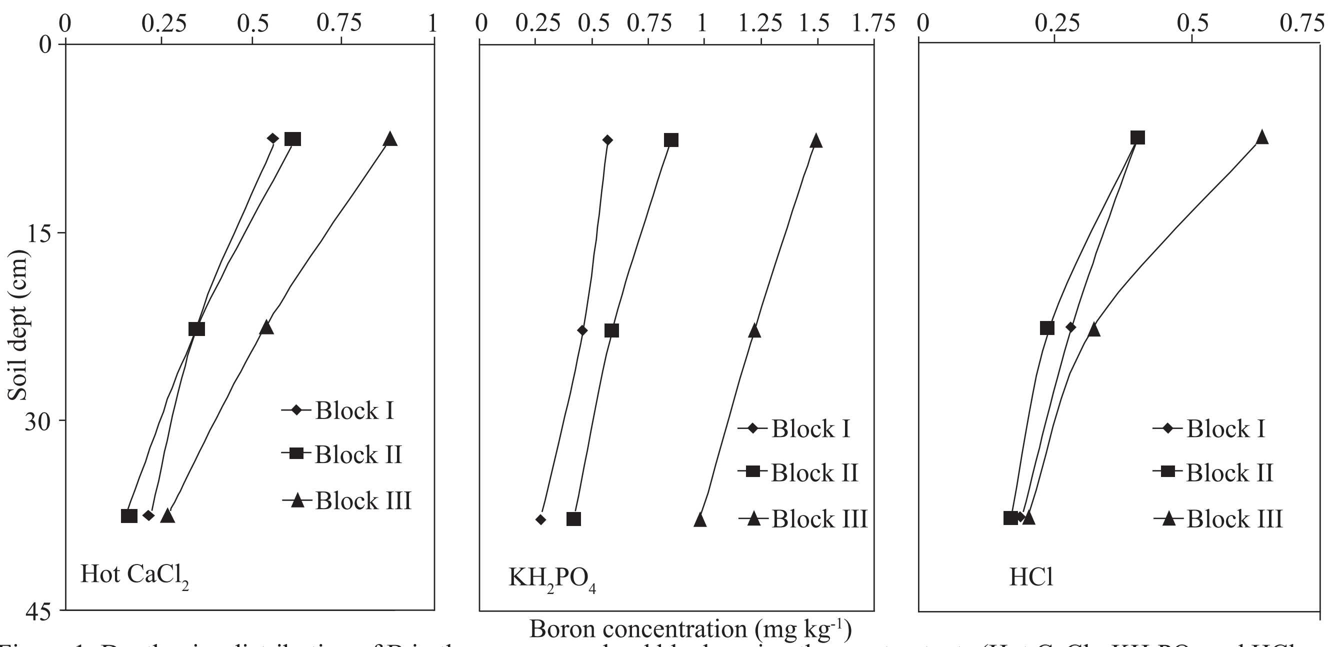

Figure 1: Depth wise distribution of B in three guava orchard blocks using three extractants (Hot $CaCl_2$, KH_2PO_4 and HCl

(Table 3). This decline in B content may be due to decrease in OC, and amorphous Fe and Al oxide content of the soils with depths. Similar results were obtained by Mandal and De, 1993 and (Sarkar et al., 2008) under different soil orders in soils of West Bengal.

3.4. Relationships between extractable soil B and soil properties

The amount of B extracted by each of the three extractants was positively and significantly correlated with oxidisable organic carbon (Table 4). This indicates that the extractable-B content of the soils will increase with increasing amount of OC. This represents that the all three extractants are known to be capable of extracting organic matter bound B (Gupta et al., 1985). Similar results were reported by (Gupta et al., 1985; Mandal et al., 2004; Sarkar et al., 2008). The B extracted using hot $CaCl_2$ and HCl as extractant are positively and significantly correlated with clay. This is due to that the two extractants are capable of extracting clay bound B and similar positive correlation

Table 4: Pearson's Correlation between extractable soil B and selected soil properties in different Guava orchard Blocks

	Hot $CaCl_2$ B	KH_2PO_4 B	HCl-B
pH	0.136	0.067	0.178
Ox. Organic Carbon	0.556**	0.517**	0.474**
Clay	0.468**	0.136	0.410**
Amorphous Fe	0.197	0.347*	0.159
Amorphous Al	0.379**	0.415**	0.236
Mn-Oxides	-0.012	-0.065	-0.115

*,** Significant at 0.05 and 0.01 probability levels, respectively

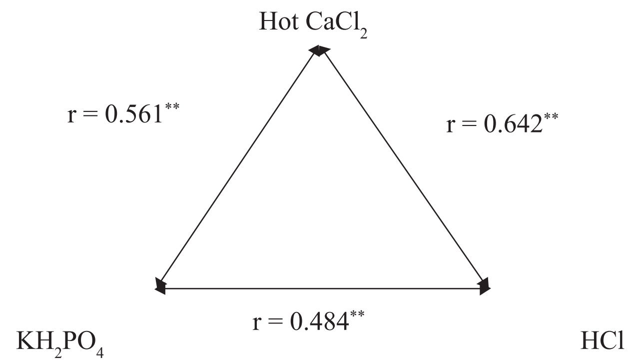

Figure 2: Dynamic relationships among extractable forms of B in soils of guava orchard blocks. (* and ** represent the significance at 0.05 and 0.01 probability levels, respectively)

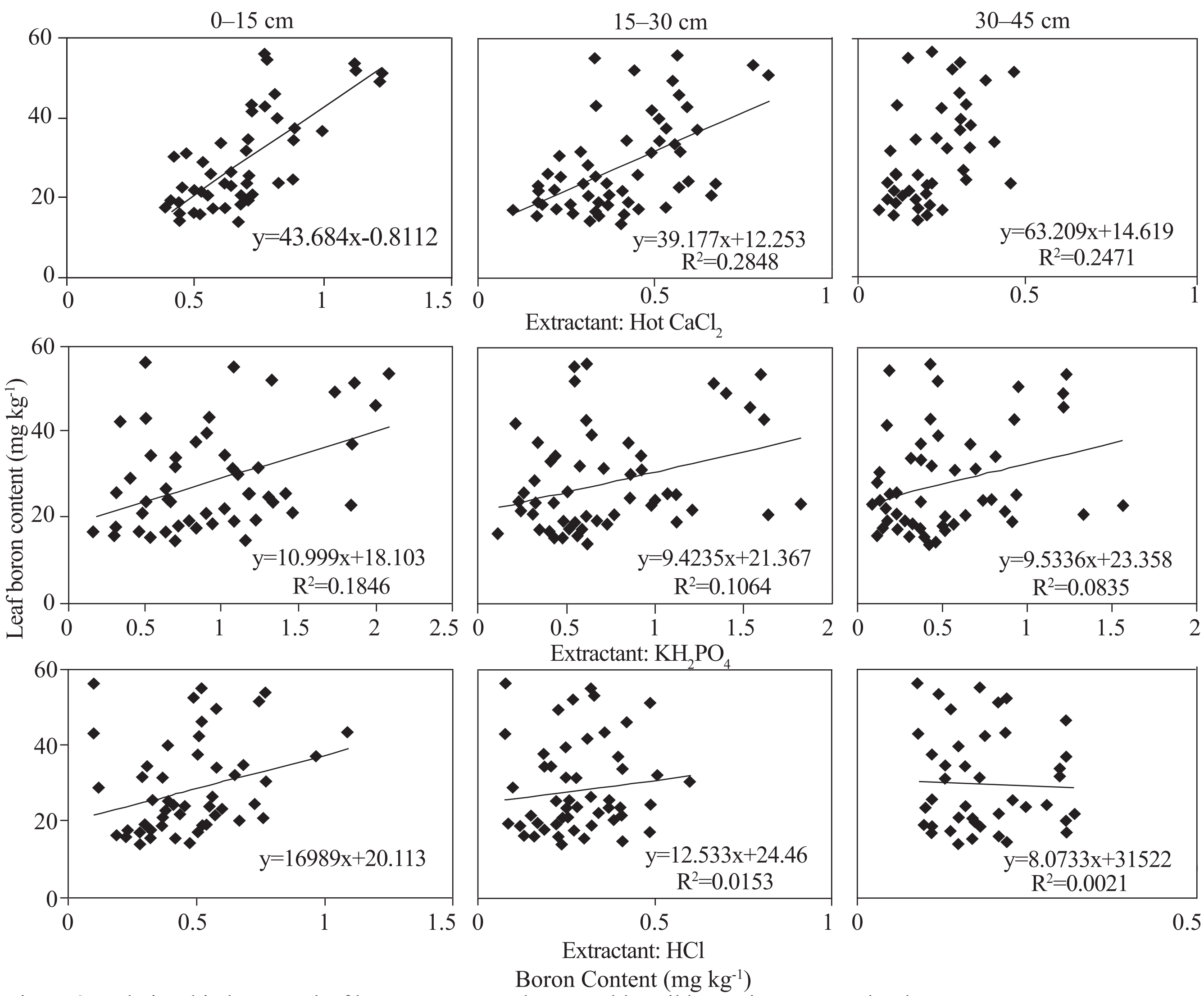

Figure 3: Relationship between leaf boron content and extractable soil boron in guava orchard

between the two parameters were also observed by Fleming, 1980; Elrashidi and O'Connor, 1982. The KH_2PO_4 extracted B has positively and significantly correlation with amorphous Fe and Al (Table 4). This explains that the KH_2PO_4 also extracts those B which are bounded with amorphous Fe and Al. Thus, increases in amount of amorphous Fe and Al results in increase in amount of B. Although the extraction of B bounded with oxides of Fe and Mn is not well known because these oxides combined with B through ligand exchange mechanisms, making it difficult to extract (Mott, 1981; Mandal and De, 1993). However, the amorphous nature of such oxides of Fe and Al when extracted with KH_2PO_4, which act as pH buffer, may help B to get released to the soil solution and accounted in the extractant B for this extracting agent. Hot $CaCl_2$ B is positively and significantly correlated with amorphous Al. This indicates that the hot $CaCl_2$ can extract amorphous Al bound B and shows the increase in amount of B release in soil solution on use of hot $CaCl_2$ as an extractant.

Figure 2. represent the dynamic relationship among extractable forms of B in soils. The amounts of B extracted by the three extractants were found to be positively and significantly correlated with each other. This indicates that the extractants extract B from more or less similar pools in the soils. The magnitude of efficiency of extractants varies with one another based on properties of the soils. Similar results were reported by (Sarkar et al., 2008) for different soils.

3.5. Relationship between Leaf B content and soil B using different extractant

Figure 3. indicates the relationship between leaf B content and soil extractants in different soil depths. The leaf B content showed weak correlation with the soil extracted B for all the three soil extractant in different depths. The availability of more B in the soil influences more uptake of B resulting into more accumulation of B in leaf. Among all soil extractant, hot $CaCl_2$ at 0−15 cm is more correlated with leaf B content with respect to other extractants alongwith the depths concerned.

The B extracted by each of the three extractants has more correlation coefficient value with available B of surface soil layer than the sub surface soil layer. This is due to the surface feeding nature of the guava crop and also the higher availability of nutrient in the surface layer enabling readily uptake and hence, higher correlation.

4. Conclusion

The study indicates that the available B content decreases with increase in soil depth. The ability of different extracting procedures to extract B is influenced by the soil characteristics even within same soil order. B content in leaf tissue has more correlation with surface soil layer than the sub surface soil layer and also hot $CaCl_2$ is more related to leaf B content in comparison to KH_2PO_4 and HCl extractants.

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