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Comparing Boron Soil Testing Methods for Coastal Plain Sandy Soils

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ABSTRACT

There are several methods for determining boron (B) in soil for soil testing purposes, yet variability among methods can lead to inconsistencies in determined concentrations for the same soil sample. The most typical method used to determine B in soil is hot-water B (HWB) extraction, but this method has reduced efficiency when dealing with large sets of soil samples along with other disadvantages such as non-uniformity in the boiling of samples. As a result, universal extractants have become increasingly popular for the extraction of B due to enhanced efficiency in a high through-put laboratory setting. This study uses a Deming regression model to compare the traditional HWB extraction method with two common universal extractants, Mehlich-1 (M1), and Mehlich-3 (M3), for coastal plain sandy soils. Results showed M3-B extractability is consistently higher than M1-B and HWB for all tested soils. The M3 method was then verified for precision using blind extension soil samples. The hot-water method was unsuccessful in producing reliable extractable B concentration in soils with high calcium carbonate and organic matter contents. Results from this study can be used to optimize B determination for high throughput production laboratory settings.

1. Introduction

Determining boron (B) in soil has been one of the most challenging and difficult soil tests routinely performed in laboratories (Mahler, Naylor, and Fredrickson 1984; Shiffler et al. 2005). Many commercial laboratories in the United States follow various extraction methods in determining B in soil. For example, a universal extractant such as ammonium bicarbonate-diethylenetriamine pentaacetate acid (AB-DTPA) is used for the analysis of B especially for calcareous soils by some laboratories (Gestring and Soltanpour 1984), while other laboratories use DTPA-sorbitol method of extraction for B on similar soils with widely ranging physical and chemical characteristics (Redd et al. 2008). Such variability in the methods can lead to inconsistencies and variations in the determined concentrations for the same soil sample because the results are dictated by the specific method employed (Liuzza et al. 2020).

The more popular methods for B determination in soil are a) hot-water boron (HWB) (Berger and Truog 1939), b) hot $CaCl_2$, mannitol $CaCl_2$ (Schuppli 1986), c) pressurized hot water method of extraction for B (PHWB) (Webb, Hanks, and Jolley 2002) and universal extractants such as d) Mehlich-1 (Mehlich 1953), e) Mehlich-3 (Mehlich 1984), f) AB-DTPA (Gestring and Soltanpour 1984), and g) DTPA-sorbitol (Mattila and Rajala 2021). These methods differ from one another in terms of their effectiveness. For example, the hot $CaCl_2$ method is mostly suited for acidic soils (Datta,

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Bhadoria, and Kar 1998; Offiah and Axley 1988; Shiffler et al. 2005). For alkaline soils, universal extractants such as AB-DTPA and DTPA-sorbitol can act as effective chelating agents for the extraction of B. The DTPA-sorbitol method has possibility for occurrence of microbial contamination when applied in routine analysis (Shiffler et al. 2005). Collectively, B determination remains inconsistent among methods, most often leading to variability between plant uptake and crop response measurements.

The HWB is the most widely used extraction method for water-soluble B in soil serving as an indicator for plant uptake (Berger and Truog 1939; Offiah and Axley 1988; Reisenauer et al. 1973). Hot-water B is commonly used because the water-soluble B extracted by hot-water method correlates positively to plant growth response as it measures B from the adsorbed, organic, and soluble pools of the soil at boiling temperatures (Offiah and Axley 1993; Sarkar et al. 2008). However, despite this method being accepted as a reliable method of extraction for B, HWB remains time-consuming, imprecise due to non-uniformity in boiling of the samples, loss of water due to evaporation, and is only applicable for B analysis, resulting in reduced handling efficiency in the lab (Dupré et al. 2019; Zbíral and Němec 2009; Sah and Brown 1997; De Abreu et al., 1994).

These challenges with the HWB extraction method make it inefficient to use this method on a regular basis in a large-scale commercial laboratory setting with a high throughput of samples. Researchers have since explored the relationship between HWB and other extraction methods to help resolve this issue (Zbíral and Němec 2009). Universal extractants Mehlich-1 and Mehlich-3 have become the most common alternative for B extraction, along with other plant nutrient elements (Dupré et al. 2019; Redd et al. 2008; Mylavarapu et al., 2002; Shuman et al. 1992). Yet, these methods have not been studied in a way that would allow for uniformity in B determination; a standard method with increased handling efficiency that can be well correlated with HWB extraction remains limited in the soil sciences. Adoption of a single multielement analysis method is expected to result in rapid and high handling efficiency for laboratories with large volumes and high throughputs of samples (Dupré et al. 2019).

The purpose of the study is to compare the popular HWB method with universal extractants Mehlich-1 and Mehlich-3 using coastal plain sandy soils to establish a standard uniform soil testing procedure that is cost-effective and rapid for the determination of B. For this study, four random soil samples were considered in which B was extracted using the three methods, HWB, Mehlich-1, and Mehlich-3. The extractable B concentrations for all three methods were statistically compared using Deming regression. The most rapid reliable method was then used on blind extension soil samples representing the coastal plain sandy soils. The results of this study can be used to optimize the method B determination in a commercial production laboratory.

Methods

Soil sampling

The study used four unique soil series samples from different locations across the state of Florida. The series description (USDA-NRCS 2021) and the locations of the soil samples collected are given in Table 1. Candler series (sandy entisol) and Tifton series (fine-loamy ultisol) are two acidic-mineral soils from the northern region of Florida. The Krome (loamy entisol) series is a typical south Florida

Table 1. Soil series, location, and classification of soils used in the study.

Series	County	Location	Classification
Candler	Marion	29.4057, −82.1393	Hyperthermic, uncoated Lamellic Quartzipsamment
Pahokee	Palm Beach	26.6618, −80.6376	Euic, hyperthermic Lithic Haplosaprists
Krome	Miami-Dade	25.5131, −80.5002	Loamy-skeletal, carbonatic, hyperthermic Lithic Udorthent
Tifton	Gadsden	30.5506, −84.7103	Fine-loamy, kaolinitic, thermic Plinthic Kandiudult

Table 2. Physical and chemical soil properties for the surface (0–15 cm) profile of different soils in the study.

Soil Parameter	Candler	Tifton	Krome	Pahokee	Method of analysis
Sand (%)	98	85.5	83	86	Hydrometer method
Silt (%)	1	13	7	9	
Clay (%)	1	1.5	10	5	
pH (1:2)	6.36	4.86	7.67	7.01	EPA[a] Modified Method 150.1
EC (dS/m)	0.06	0.07	1.04	2.89	EPA[a] Modified Method 120.1
CEC (meq/100 g)	2.36	3.76	76.42	110.45	Calculation
SOM[b] (%)	0.69	2.54	5.48	73.39	Walkley-Black/LOI[c]
HWB (mg/kg)	0.08	0	0.16	-	EPA[a] Modified Method 200.7
M1-B (mg/kg)	0.01	0	0.18	1.82	
M3-B (mg/kg)	0	0.06	1.33	4.76	

a = Environmental Protection Agency, b = Soil Organic Matter, and c = Loss on ignition

calcareous soil, where the marl (calcium carbonate "mud') and rocky portions of soils comprise nearly 30% to 94% calcium carbonate (Mylavarapu, Harris, and Hochmuth 2019). The Pahokee series is an organic muck histosol located in southern Florida. Samples were collected at random locations in the field from depths of 0–15 cm from the surface soil, air-dried (100°F), ground and passed through a 2 mm sieve.

All soil samples were analyzed for physical and chemical properties of soil such as texture, pH (1:2 soil:water), electric conductivity (EC), organic matter content, and cation exchange capacity (CEC) using standard reference methods. Their characteristics are provided in Table 2. These five soil samples were analyzed for B concentrations using three extraction methods: HWB, M1, and M3. All the three solutions were quantified for B by Inductively coupled plasma-optical emission spectrometry (ICP-OES) (Spectro Arcos (2), Spectro Analytical Instruments, Mahwah, NJ 07430 S/N 15006641) at the wavelength of 249.773 nm.

Soil characteristics

Based on the standard reference methods used to determine physicochemical properties, the pH of the soil samples ranged from 5.30 to 7.67 for the four soils in the study (Table 2). The pH of the calcareous soil (Krome) with high calcium carbonate content was the highest (7.67), followed by organic soil (Pahokee) with a neutral pH (7.01) and acidic-mineral soils, Candler, and Tifton with a pH of 6.36, and 4.86, respectively (Table 2). The textures of the soils were sandy (Candler), loamy sand (Tifton), sandy loam (Krome), and muck (Pahokee). The organic matter content of the soils was less than 2% in the Candler series and more than 2% in the Tifton and Krome series, at around 2.54% and 5.48%, respectively, with the Pahokee muck series having the highest organic matter (73.39%) (Table 2). Applied to samples of each soil series were 11 different treatment concentrations from a 100-ppm primary B standard. The resulting soil B concentrations were: 0 (control), 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5-mg/kg. Each treatment was replicated 5 times, and the entire study was repeated three times.

Laboratory protocol and quality control

Operations within an analytical laboratory must address quality control issues to maintain both accuracy and precision. The method detection limit (MDL) determined by the laboratory for B analysis by ICP-OES at 249.773 nm is 0.0007 mg/kg. The minimum reporting limit (MRL) or practical quantitation limit (PQL) is 0.05 mg/kg (Mylavarapu et al. 2021). The soil:extractant ratios are specific to each extraction method, and consequently appropriate dilution factors were used to calculate the final concentrations of B. The MRL for HWB, M1, and M3 methods are 0.1, 0.2, and 0.5 mg/kg B, respectively. Prior to the experiment, a method blanks study was conducted to establish the quality control for the extraction methods. Method blanks were analyzed for the three extraction

methods: HWB, M1, and M3 with an objective to determine any sources of contamination from glassware, plasticware, or filter paper used in the analysis. A method blank is a blank solution with the extractant (hot-water or M1 or M3) in study, which is subjected to the same extraction process involved for the analyte in study (B) but without the soil sample. The assessment of method blanks is critical when analytes in soil extracts are measured at low concentrations, especially, micronutrient, heavy metal, or herbicide analysis. All the three methods along with method blanks were quantified for B by ICP-OES instrumentation.

Hot-water boron

Soil samples (10-g) were weighed into 125-ml Erlenmeyer glass flasks using a calibrated analytical balance (Mettler PE 3600). Twenty milliliters of deionized (DI) water was added to the weighed soils using a measuring cylinder or a calibrated dispenser. Small glass funnels were mounted over the Erlenmeyer flasks to aid in the reflux. The flasks were placed on a heated hot plate and observed until boiling. Once boiling, samples remained boiling for 10 min. The boiled soil solution was then transferred into plastic cups using Whatman No. 1 filter paper. The extracted filtrate was transferred into plastic scintillation vials. Refer to Berger and Truog (1939) for more information about HWB.

Mehlich-1

Soil samples (5-g) were weighed into glass containers using a calibrated analytical balance (Mettler PE 3600). Twenty milliliters of M1 extractant [0.0125 M sulfuric acid (H_2SO_4), 0.05 M hydrochloric acid (HCl)] were added to the weighed soil using a calibrated dispenser. The soil solution was covered with plastic lab wrap and shaken using an end-end mechanical shaker for 5 minutes. The extracted solution was filtered using Whatman No. 1 filter paper. The filtrate was transferred into scintillation vials. Refer to Perkins (1970) for more information about M1.

Mehlich-3

Soil samples (2.5-g) were weighed in plastic containers using a calibrated analytical balance (Mettler PE 3600). The use of plastic labware is an important step for this extraction method to avoid B contamination in the solution as one of the M3 reagents [Ammonium Fluoride (NH_4F)] causes etching of glass and, hence, release of B from standard laboratory borosilicate glass containers. Twenty-five milliliters of M3 extractant [0.2 M acetic acid (CH_3COOH), 0.015 M ammonium fluoride (NH_4F), 0.25 M ammonium nitrate (NH_4NO_3), 0.013 M nitric acid (HNO_3), 0.001 M ethylenediami-netetraacetic acid (EDTA)] were added to the weighed soil using a calibrated dispenser. The soil solution was covered with plastic lab wrap and mechanically shaken for 5-min. The extracted solution was filtered using Whatman No. 1 filter paper. The filtrate was transferred into scintillation vials. Refer to Mehlich (1984) for more information about M3.

Statistical analysis

All data were analyzed using linear mixed model methodology as implemented in SAS® PROC MIXED (SAS/STAT 15.1; SAS Institute, Cary, NC).

Regression analysis for recovered B on applied B

The relationship between applied and recovered B was modeled using linear regression with rate applied nested within each soil (eq.1):

$$Boron = Soil + Rate\,(Soil) \tag{1}$$

where Boron represents the amount of B recovered, Soil refers to the five soil types used in this study, and Rate is the amount of B applied. The three extraction methods also included experimental repeats with the treated samples to examine consistency of the method. The experimental repeats were evaluated for percent recovery efficiency for each soil and method. Experimental repeat was considered a random blocking effect. To account for heterogeneous residual variances, variance groups based on the interaction of {soil × method} combinations were created. Least squares mean and 95% prediction intervals were calculated and plotted along with the interaction of {soil × rate} averages.

Equation used for evaluating precision by calculating B recovery percentages on applied B for three methods for all soils in study was:

Percent B recovery calculation:

$$\frac{[\text{Actual B recovered } - \text{ Native B concentration in the soil}] \times 100}{\text{Actual B applied}}$$

For quality control purposes, precision is expressed as relative percent difference (RPD). The desirable recovery for an analyte in a laboratory experiment is between 90% and 110% (Mylavarapu et al. 2020b; US EPA 2018).

Comparison of extraction methods using Deming regression

A Deming regression was used to compare extraction methods because both variables, e.g., method 1 (m1) and method 2 (m2), are measured with error; standard linear regression models assume the independent variable X to be measured without error. As such, a regular linear regression analysis in such situations would generally underestimate the intercept while overestimating the slope. For each soil and pair of methods, we used the model (eq.2):

$$\text{Boron}_{m1} = \text{Boron}_{m2} \tag{2}$$

where Boron is the amount of B measured and m1 and m2 refer to the two methods (method-1 and method-2) being compared. Intercept and slope were estimated using SAS/IML® 15.1 (SAS Institute, Cary, NC) routines. Standard errors for intercept and slope estimates were derived via the jack knife procedure as recommended by Linnet (1993).

Verification of the method

The most optimum (i.e., consistent, reliable) extraction method, based on the results of recovered B, was chosen as a standard procedure to further determine B in soil. To evaluate and establish the method as robust, a verification study was conducted using blind extension soil samples. A total of 263 soils with wide pH ranges from various counties across the state assembled at the UF/IFAS Extension Soil Testing Laboratory (ESTL) were sampled for B concentration using the optimized extraction method and were analyzed by ICP-OES at 249.773 nm wavelength. Out of the 263 soil samples, 122 soil samples represented home landscapes and vegetable gardening from 33 counties across the state (Figure 1), while the rest of the 141 samples represented commercial farms typically sandy soils from 21 counties across the state (Figure 1). These soil samples were examined for quality control and quality assurance meeting the laboratory standards. For every 20 samples, one sample was replicated, and RPD was calculated to determine the precision.

Results

The quality controls meeting the laboratory standards were assessed and maintained for all three extraction methods: HWB, M1, and M3 throughout the experiment. The method blanks for the extraction methods were analyzed, and it was observed that the use of the glassware for M3 extraction
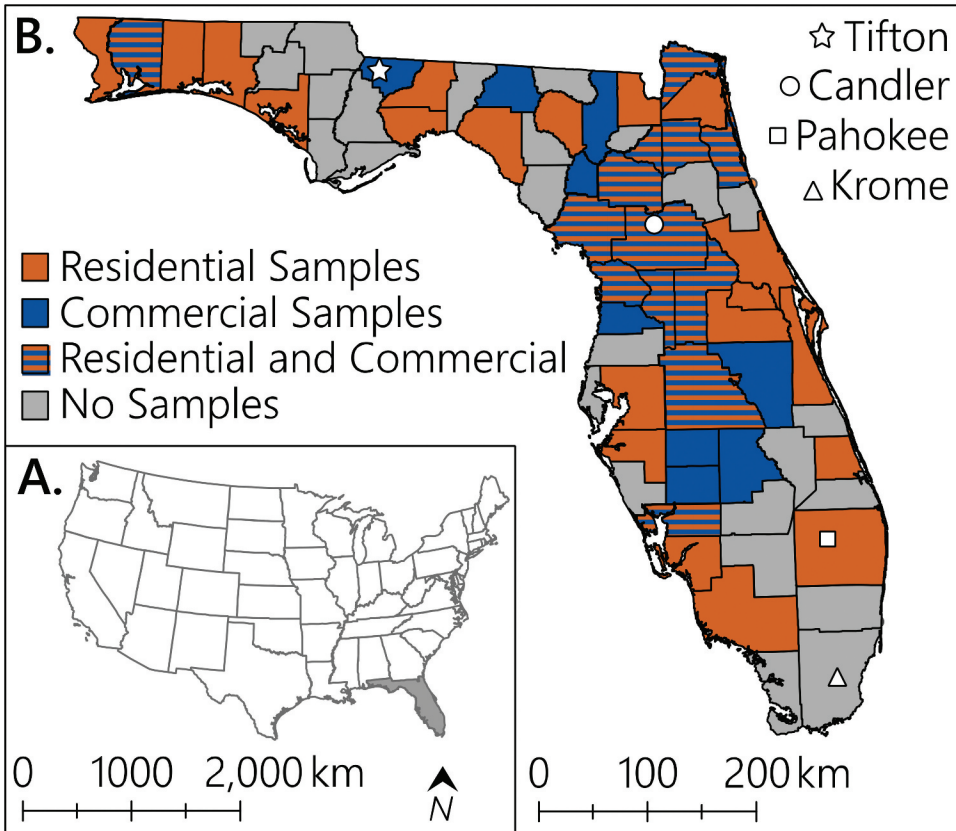
Figure 1. Map of Florida representing four major soils in study and blind extension soil samples received at the laboratory from various counties across the state.

method is not suitable as NH_4F in M3 extractant could possibly leach B to the soil solution due to etching of glass. Therefore, use of plastic labware certified to be clean or non-detect for B is a prerequisite for M3 extraction method for B analysis.

Extractable B concentrations in soils

Hot-water boron

Results for recovered B versus applied B using the HWB method on acid-mineral (Candler and Tifton) and calcareous (Krome) soil are displayed in Figure 2.

    The amount of B extracted from samples with known rates of B applied was highly variable for different soils; for Candler series, the recovery ranged from 40% to 160% (Figure 2) with large difference between the two repeats. For Tifton and Krome series, the amount of extracted B recovery ranged from 10% to 60% across the different applied B rates. The replicates for each B rate were highly spread out for acid mineral soils (Candler and Tifton). For example, within the five replicates of samples spiked with 1.5 mg/kg B, the recoveries range between approximately 50–90% for one repeat, while around 140–160% for another repeat where overestimation of percent B recovery was observed in the case of Candler series. Similarly, for Tifton series, for the 1 mg/kg applied B rate, the recoveries ranged between 10% and 60% in one of the two repeats. On the other
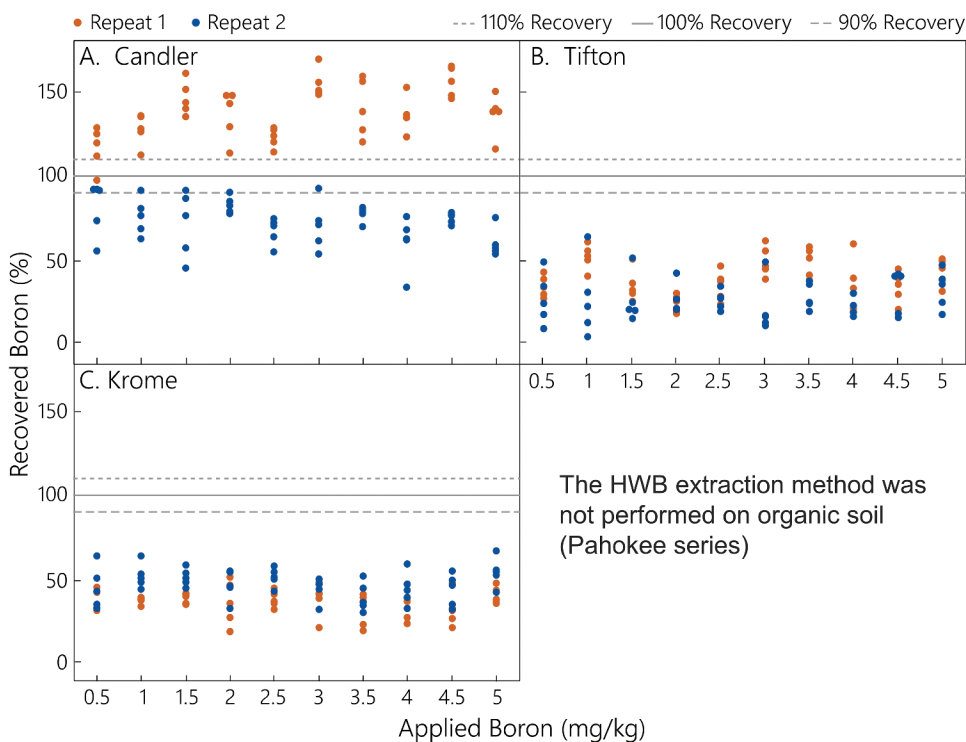
Figure 2. Evaluating recovery efficiency of experimental repeat for HWB extraction from Candler, Tifton, and Krome series soils. The dots represent the actual B values measured for applied B rate with two experimental repeats. There are five replicates for each B rate for each repeat.

hand, although the percent recovery is low, calcareous (Krome) soil has approximately ± 15% variation between repeats, which is relatively more precise than acid-mineral soils in this case (Figure 2).

Mehlich-1 extractable boron

Results for recovered B using M1 for acidic-mineral (Candler and Tifton), calcareous (Krome), and organic (Pahokee) soil are displayed in Figure 3.

The observed recovery percentages for the M1 extraction method were roughly 75–90% for the acid mineral (Candler and Tifton) soils and approximately 50–65% for calcareous (Krome) and organic (Pahokee) soil (Figure 3). The recovery of B increased from approximately 45–60% with the increase in applied B for Krome series. For Tifton series, the recoveries were less than 60% for the lower spike level of 0.5 mg/kg, while the percent recovery increased between 60% and 80% as the rate of B applied increased in one of the repeats. The replicates for each B rate for each repeat are precise for all soils in study except in Pahokee series where in one of the repeats the replicates of 3 mg/kg applied B rate had inconsistent B recoveries but not excessive variation. The variability in the B recoveries among the three repeats was not large for Candler, Krome, and Pahokee series, while for Tifton, the B recoveries were considerably different from one repeat to another (Figure 3). The desirable precision with 90% recovery was observed in one of the repeats for acid-mineral soils (Candler and Tifton).

Mehlich-3 extractable boron

Results for recovered B using M3 on applied B for acid-mineral (Candler and Tifton), calcareous (Krome), and organic (Pahokee) soil are displayed in Figure 4.
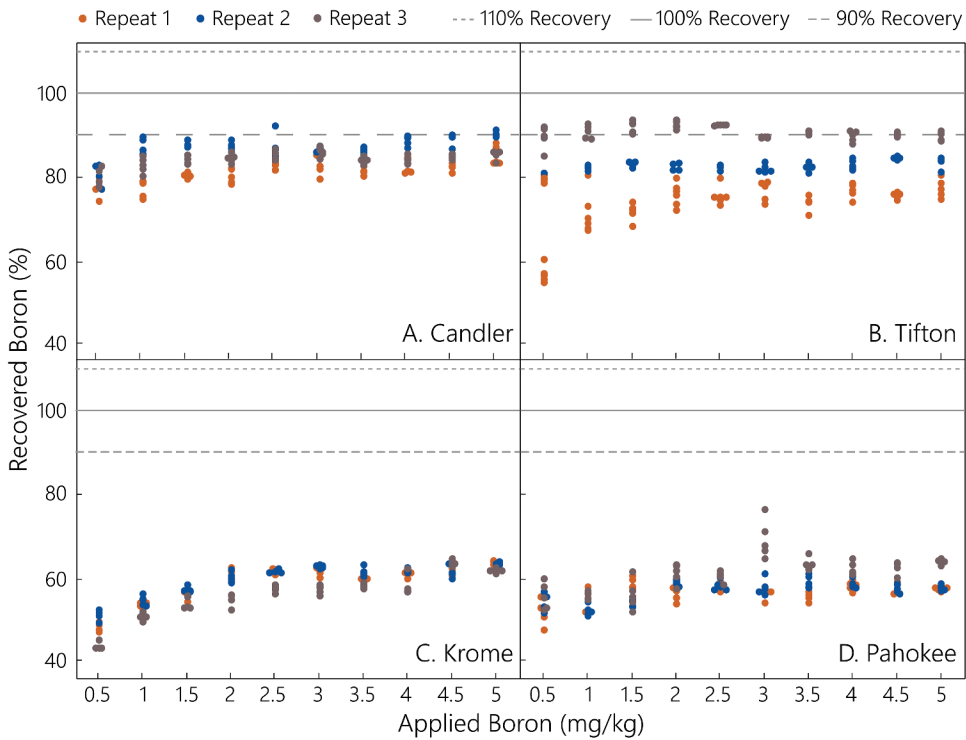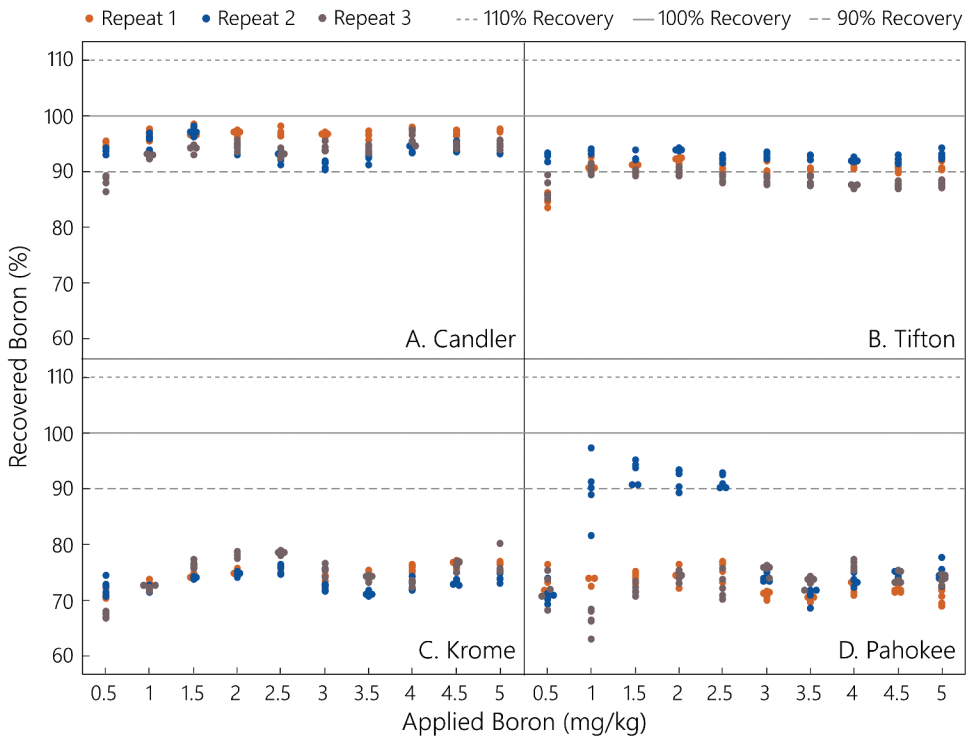
Figure 3. Evaluating recovery efficiency of experimental repeat for M1-B extraction from Candler, Tifton, Krome, and Pahokee series soils. The dots represent the actual B values measured for applied B rate with three experimental repeats. There are five replicates for each B rate for each repeat.

The observed recovery percentages for the M3 extraction method were approximately 90% recovery of the applied B for acid-mineral soils (Candler and Tifton) and approximately 70–75% recovery for both calcareous (Krome) and organic (Pahokee) soil (Figure 4). The percent B recovery for acid-mineral (Candler and Tifton) soils was in the desirable range between 90% and 110% except for the lower applied B rate of 0.5 mg/kg where the recoveries were relatively low with approximately 85–90%. The replicates for each applied B rate for each repeat are precise for all soil types except in Pahokee series where one repeat had inconsistent B recoveries for lower B rates from 1 mg/kg to 2.5 mg/kg. The variability in the B recoveries among the repeats was not excessive for Candler, Tifton, and Krome series (Figure 4).

Comparison of regression analysis for three extraction methods

The slope estimates measuring the relationship between recovered B on applied B rates for the soils using linear regression model are provided in Figure 5.

The slopes for the three respective methods in Figure 5 are linear. The slope estimate values in Table 3 are referring to the recovery efficiency of B to the applied B rate for the soils along with 95% confidence interval. The slopes estimates were different for each method as the dilution factor (soil: extractant ratio) is different for all the three methods in study. The dilution factor for HWB, M1, and M3 are 2, 4, and 10, respectively. The results show that the slope estimates for M3 for acid-mineral (Candler and Tifton) were 9.55 and 9.01, respectively, implying approximately 95% and 90% of the applied B were recovered for Candler and Tifton series soils, respectively (Table 3). For calcareous (Krome) and organic (Pahokee) soil, the M3 slope estimates were 7.52 and 7.25, implying approximately 75% and 73% of the applied B were recovered for Krome and Pahokee series soils, respectively.

Figure 4. Evaluating recovery efficiency of experimental repeat for M3-B extraction from Candler, Tifton, Krome, and Pahokee series soils. The dots represent the actual B values measured for applied B rate with three experimental repeats. There are five replicates for each B rate for each repeat.

Similarly, the slope estimates for M1 for acid-mineral (Candler and Tifton) were 3.45 and 3.34, respectively, implying approximately 86% and 83% of the applied B were recovered for Candler and Tifton series soils, respectively (Table 3). For calcareous (Krome) and organic (Pahokee) soil, the M1 slope estimates were 2.50 and 2.39, implying approximately 63% and 60% of the applied B was recovered for Krome and Pahokee series soils, respectively. Furthermore, the slope estimates for HWB for acid-mineral (Candler and Tifton) were 2.10 and 0.70, respectively, implying approximately 105% and 35% of the applied B were recovered for Candler and Tifton series soils, respectively. For calcareous (Krome) soil, the HWB slope estimate was 0.81, implying approximately 40% of the applied B was recovered for Krome series soil. On the other hand, the intercept values indicate the native soil B concentration. The results show that the extracted native soil B concentrations were not different from one method to another in the case of acid-mineral soils (Candler and Tifton). On the contrary, the intercepts for calcareous (Krome) and organic (Pahokee) soil were different for M3 and M1 (Krome and Pahokee), and M3 and HWB (Krome). For acid-mineral (Candler and Tifton) soils, the B intercept values were negligible, below the MRL (0.5 mg/kg) for HWB, M1, and M3. The M3-B intercept values for calcareous (Krome) and organic (Pahokee) soil were 1.20 and 5.33, respectively, while, for M1-B, the intercept was negligible for Krome series and was 1.70 for Pahokee series (Table 3).

Deming regression

For comparison of methods using Deming regression, slope estimates explaining the ability of one method to predict the outcome of another method are provided in Table 4.
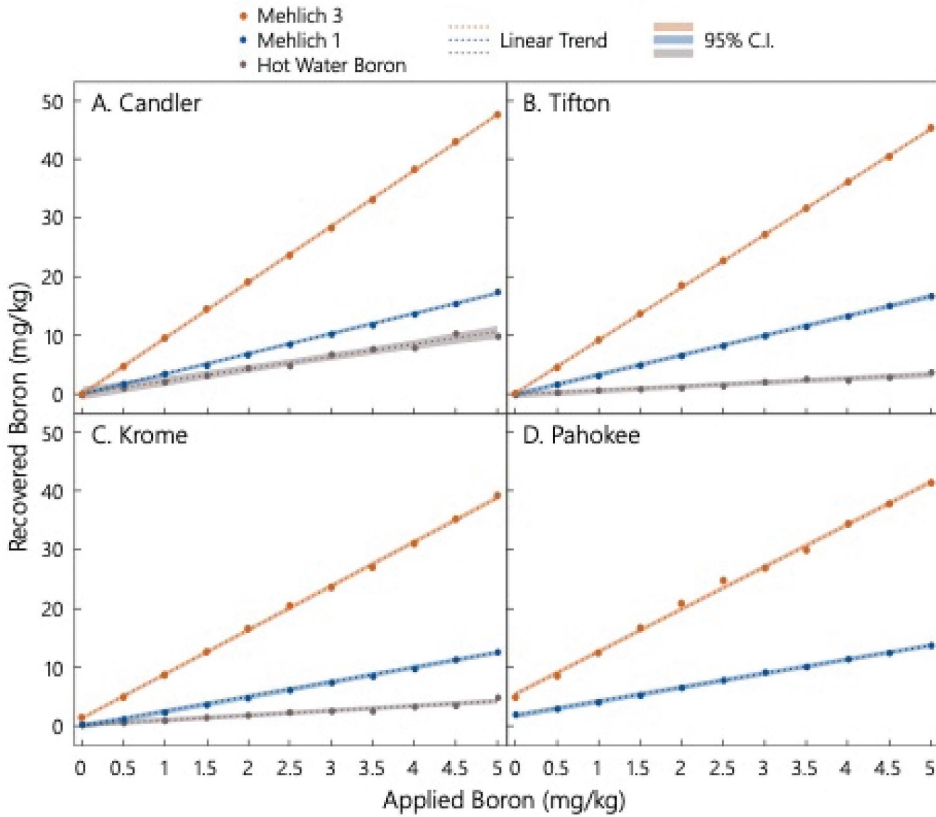
Figure 5. Linear regression of recovered B on applied B for Candler, Tifton, Krome, and Pahokee series soils for the three extraction methods HWB, M1, and M3. The line represents the predicted values and the shaded area the 95% prediction interval.

Table 3. Intercept and slope estimates for the three methods in study denoted with letters 'a,' 'b,' 'c' for any significant mean differences. The values in brackets represent the 95% confidence interval limits.

Method	Intercept (95% CI)		Slope (95% CI)	
		Candler		
M3	−0.12 (−0.45, 0.22)	a	9.55 (9.49, 9.60)	a
M1	−0.14 (−0.45, 0.18)	a	3.45 (3.42, 3.48)	b
HWB	0.05 (−0.87, 0.98)	a	2.10 (1.80, 2.40)	c
		Tifton		
M3	0.11 (−0.22, 0.43)	a	9.01 (8.97, 9.06)	a
M1	−0.10 (−0.47, 0.27)	a	3.34 (3.26, 3.42)	b
HWB	−0.20 (−0.61, 0.21)	a	0.70 (0.60, 0.80)	c
		Krome		
M3	1.20 (0.87, 1.54)	a	7.52 (7.46, 7.58)	a
M1	−0.15 (−0.46, 0.16)	b	2.50 (2.48, 2.53)	b
HWB	0.10 (−0.26, 0.46)	b	0.81 (0.74, 0.89)	c
		Pahokee		
M3	5.33 (4.96, 5.70)	a	7.25 (7.17, 7.33)	a
M1	1.70 (1.39, 2.01)	b	2.39 (2.37, 2.41)	b

Table 4. Intercept and slope estimates from the variable in regression (Deming regression) comparison of three extraction methods in four soils in study. The values in square brackets represent the 95% confidence limits.

		Y-variable	
Soil/ Parameter	X–variable	Mehlich 1	Hot water Boron
Candler			
Intercept	Mehlich 3	−0.08 [−0.16, 0.01]	−0.00 [−0.61, 0.60]
Slope	Mehlich 3	0.36 [0.36, 0.37]	0.22 [0.19, 0.26]
Intercept	Mehlich 1		−0.10 [−0.89, 0.70]
Slope	Mehlich 1		0.64 [0.51, 0.77]
Tifton			
Intercept	Mehlich 3	−0.11 [−0.26, 0.04]	−0.06 [−0.25, 0.12]
Slope	Mehlich 3	0.37 [0.36, 0.38]	0.08 [0.07, 0.09]
Intercept	Mehlich 1		−0.07 [−0.28, 0.14]
Slope	Mehlich 1		0.21 [0.18, 0.24]
Krome			
Intercept	Mehlich 3	−0.54 [−0.61, −0.47]	−0.01 [−0.20, 0.18]
Slope	Mehlich 3	0.33 [0.33, 0.34]	0.11 [0.10, 0.12]
Intercept	Mehlich 1		0.16 [−0.02, 0.34]
Slope	Mehlich 1		0.33 [0.29, 0.36]
Pahokee			
Intercept	Mehlich 3	0.01 [−0.08, 0.10]	
Slope	Mehlich 3	0.33 [0.32, 0.33]	

A Deming regression approach was used to explain the ability of one method to predict the outcome of another method (Table 4). Results show that when HWB was regressed on M3 for comparing the two methods, M3 extracted more B than HWB (Table 4). The slope estimates for acid-mineral soils were 0.22 and 0.08 for Candler and Tifton, respectively. For calcareous (Krome) soil, the slope estimate was 0.11. The variability of slopes was wide for HWB method for the three soils implying that the method is very inconsistent. The results show that when M1 was regressed on M3 for comparing the methods, M3 extracted almost three times more B than M1 (Table 4). The slope estimates for acid-mineral soils were 0.36 and 0.37 for Candler and Tifton, respectively. The slope estimate was 0.33 for both calcareous (Krome) and organic (Pahokee) soil. The variability of slopes is narrow for these two methods implying that the method is very consistent and reliable. The results show that when HWB was regressed on M1 for comparing the methods, M1 extracted more B than HWB (Table 4). The slope estimates for acid-mineral soils were 0.64 and 0.21 for Candler and Tifton, respectively. For calcareous (Krome) soil, the slope estimate was 0.33. The variability of slopes is wide again for HWB method implying that the method is very inconsistent and not reproducible.

Method verification study

Figure 6 provides B concentrations for blind extension soil samples grouped by pH ranges for commercial farm, and landscapes and vegetable home garden soils, respectively.

These data were verified using client samples submitted to the UF/IFAS Extension Soil Testing Laboratory (ESTL) from across the state by extracting with the standard M3 extraction method. The soil samples were grouped based on the pH ranges. The minimum reporting limit (MRL) setup by ARL, UF/IFAS for B analysis by ICP-OES with 99% confidence interval is 0.5 mg/kg for M3 extraction method. It was found that acid to neutral (≤7) pH soils of commercial farms across the state had no extractable B (zero) concentrations present in soil (Figure 6). On the other hand, soils with higher pH (>7) possessed some amount of extractable B concentrations ranging from 0 to 1.60 mg/kg B with majority B concentrations measured falling below the MRL. Similar results were obtained for landscapes and vegetable gardening soil samples. Out of the 122 soil samples, based on the pH values, it was observed that only five acid-
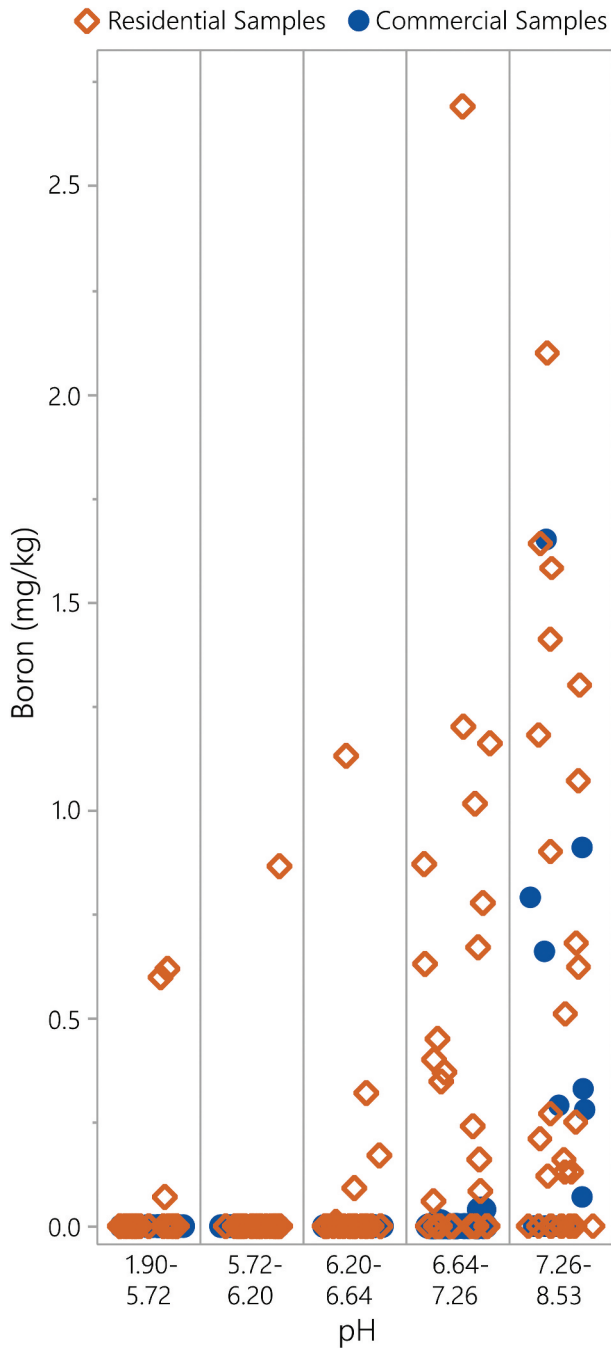
Figure 6. Extractable M3-B concentrations (in mg/kg) in extension soil samples grouped by commercial farm and residential soil samples with wide pH ranges.

mineral soils with a pH range of 1.9–7.0 had B concentrations between 0.5 and 1.25 mg/kg (Figure 6) and few other soils with the pH greater than 7 extracted about 0.5–2.8 mg/kg B concentrations (Figure 6).

Discussion

Comparison of methods

These findings are consistent with prior research investigations where M3 extracted more B than the other two methods, HWB, and M1 (Behera et al., 2016; Hopkins et al. 2010; Redd et al. 2008; Shuman et al. 1992). Our experimental methodology differs from others in that we did not use incubation periods nor a crop study to test B recovery and uptake. Instead, our primary goal was to test if the methods worked consistently in a laboratory setting producing reliable results. The reason for not including the incubation period into this study is that it is better explained when it is coupled with plant uptake in real field conditions, which laboratory incubation studies cannot replicate (Hochmuth and Hanlon 2019). In all four soils, the amount of extractable B measured by each of the three extraction methods was different due to the soil: extractant ratio, with the dilution factors of 2, 4, and 10 applied to HWB, M1, and M3 extraction methods, respectively. This is evident from the different slopes for the three methods for each soil, where M3 > M1 > HWB (Figure 5).

Although the use of M1 and M3 extractants for B is limited to acidic, and neutral to acidic soils, respectively, as demonstrated by Redd et al. (2008), in this study, M3 was observed to be successful in the wide range of pH (>7) soils including calcareous soils. Studies reported that M3 gave more extractable B than hot water because the acetate and fluoride anions in M3 could efficiently displace B from certain sorption sites (Seth et al. 2018; Zbíral 2016). Based on our results, M3 is more efficient and consistent than HWB and M1 for acid-mineral, calcareous, and organic soil in the study. The extraction capacity of M3 is high due to the presence of EDTA in the extractant that chelates the micronutrient from the soil (Mylavarapu et al. 2020). The extractable B content by M1 is more than hot water because of the presence of dilute acids like hydrochloric (HCl) and sulfuric acids (H_2SO_4) that pull B from the inorganic soluble, organic, adsorbed inorganic pools along with the soluble pools of soil (Behera et al., 2016). Furthermore, based on our findings, plastic labware is recommended for M3-B analysis, which is a critical step in the extraction process. Glassware is not recommended for the M3 extraction method as overestimation of B occurred when M3 method blanks were analyzed due to the presence of ammonium fluoride (NH_4F) in M3 extractant that could result in etching of glass leading to B leaching in the sample solution (Allen et al. 2005).

Comparison of soils and soil characteristics to B availability

The results in this study demonstrated that the recovery of applied B is lower in calcareous (Krome) and organic (Pahokee) soils due to the physical and chemical properties leading to B fixation in these soils. There are a variety of chemical and physical properties under different soil conditions that can reduce or increase the availability of B. Soil properties such as clay, organic matter, iron, aluminum, manganese oxides concentration, pH, and CEC affect the recovery and availability of B (Sarkar et al. 2008; Seth et al. 2017). Other research revealed the ability of each method to extract B in different soils was dependent on the soil reaction (pH), because in an alkaline solution, B forms insoluble compounds (primarily calcium and magnesium borates), whereas in an acidic soil, B is adsorbed by Fe, Al, and Mn compounds (Dhaliwal et al. 2019; Keren 1996). These chemical properties can impact negatively on nutrient assessment, particularly in B analysis. The appropriateness of various extractants for measuring plant-accessible soil nutrient is determined by the nature of the soil and crop type (Seth et al. 2018). Furthermore, Mandal et al. (2016) found that the amount of B nutrient element in the soil is determined by the soil-forming properties as well as the surrounding weathered rock components.

The native acid-mineral (Candler and Tifton) soils in the study were deficient in B as the coarse sandy soils are prone to B leaching due to high rainfall and high temperatures. The nature of B deficiency in coarse textured sandy soils is extensively mentioned in literature as a result of leaching losses in areas of high rainfall and temperatures (Malhi et al. 2003; Niaz et al. 2002). The acid-mineral

soils are low in organic matter and clay content, increasing B mobility in soil leading to nutrient leaching. The acid-mineral (Candler and Tifton) soils in the study have low clay content of 1% and 1.5%, respectively, and low organic matter contents of 0.69% and 2.54%, respectively. Therefore, since there are no active adsorbing sites in these soils, the nutrient is leached from the surface soil. The calcareous (Krome) soil in the study has low recoveries of applied B, possibly due to greater B adsorption in these soils because calcium carbonate acts as an important sink for B leading to formation of insoluble compounds such as calcium borates (Elseewi and Elmalky 1979; Goldberg and Forster 1991). On the other hand, organic matter is an important source for B (Okazaki and Chao 1968), Pahokee, organic muck soil used in our study possesses high concentration of native soil B (4.76 mg/kg M3-B). However, the recovery of the applied B is lower as possibly B is being adsorbed by the organic matter, which is about 74% in soil. Generally, the adsorbed B is subsequently made available to the plants through microbial decomposition of the organic matter (Bilen, Bilen, and Bardhan 2011).

Mehlich-3 verification

Based on our results from random blind extension soil samples, M3 is capable of extracting B in the soils with high to neutral pH condition. The soil samples represented a wide range of pH from across the state. Most of these soils have sandy marine deposits as their parent material with high calcium carbonate content limiting plant available B in soil. The acid-mineral soils are deficient in B due to high leaching losses. Therefore, the soils below the pH 7 in study are deficient in B. However, further studies are needed to study the crops grown on these soils and their response to the B concentration in soil. Depending upon the B availability, nutrient recommendations can be made. Furthermore, correlations between M3-B and plant B uptake can be made by including pH, organic matter, and clay content in the regression equations for analysis. Further studies should focus on confirming the effectiveness of M3 in predicting the need for supplemental B applications to the soil through field calibration studies.

Application in industrial labs

Soil testing is used by farmers and crop advisors to assess soil nutrient status and make nutrient recommendations, but there is no clear consensus on which method should be used (Hochmuth and Hanlon 2019; Mattila and Rajala 2021). Based on our results, the M3 extractant is consistent in producing reliable results with reduced variability. Mehlich-3 is becoming more widely used in soil analysis as it involves rapid determination with simple steps involved (Zbíral 2016). It is capable of analyzing a wide range of nutrients using a single universal extractant which minimizes analysis expenses and ensures results are comparable to those obtained from other traditional soil tests. Inclusion of B in M3 extraction method will widen the scope of analytical analysis. Also, the labware that is required for M3 extraction method is plasticware, which provides easy handling unlike glassware, which can break, and is economical and disposable for a high sample throughput analysis.

Challenges and limitations

Although M3 is advantageous, precise, and efficient in analyzing B in soil, this study is limited to laboratory analysis for the physiographic coastal plain sandy soils. Further research is needed to confirm the efficacy of the M3 extractant for B analysis in soil to predict plant uptake and growth by conducting field calibration and correlation studies. The HWB extraction could not be performed on organic (Pahokee) soil in study as the soil:extractant ratio was insufficient to extract a minimum of 5 mL extract for analysis by ICP-OES. Future research can focus on adjusting soil:extractant ratios for B analysis in soils with high organic matter content. Since B is mobile in sandy soil,

additional research such as column studies for leaching losses can better explain B accumulation down the soil profile and the applicability of B soil testing for sandy soils in areas with high precipitation and temperatures.

Conclusions

Methods of B extraction in soil were analyzed using samples representing the physiographic coastal plain region of the US. We conclude:

(1) Based on the results from recovery efficiency, comparison of the extraction methods using linear regression model, and Deming regression analysis, Mehlich-3 extraction method is precise, efficient, reliable, and consistent across wide pH ranges for different soils.
(2) Use of M3 proves to be advantageous as it involves rapid B determination by ICP-OES along with multi-element analytical ability. The robustness of M3 extractant is useful for a commercial laboratory setup with high throughput of samples on a daily basis.
(3) The use of glassware for M3 method overestimates B concentrations due to B leaching from the glass, potentially leading to wrong interpretations. Use of plastic labware certified to be clean or non-detect for B starting from storing the extractant to performing the extraction methodology is critical to avoid B contamination using M3 extraction method.
(4) The results indicate that the extractable B concentration is governed by various physical and chemical properties in acid-mineral soils, calcareous soils, and soils with high organic matter content.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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