



## **Evaluation of Different Extractants for Boron Estimation in Soils of Odisha and Andhra Pradesh**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

The present study was conducted to compare the four different extractants widely used in boron (B) determination in soils and to screen the most suitable extractant for acidic (Alfisols) and alkaline (Vertisols) soils of Odisha and Andhra Pradesh. A total of 200 surface soil samples were collected across two sites (100 from each site) representing different pH ranges. Hot-water-soluble boron (HWS-B) extraction procedure being the most widely used B determination procedure was kept as a benchmark in order to compare the B extracting efficiency by the extractants viz., 0.01 M Calcium Chloride (CaCl<sub>2</sub>), 1 N Ammonium acetate (NH<sub>4</sub>OAc) pH-7.0 and 0.01 M Barium Chloride. The mean values of hot water extractable B for acidic soils were 0.49 (mean between 0.18 and 1.50 mg kg<sup>-1</sup>), CaCl<sub>2</sub> – 0.42 (mean between 0.14 and 1.52 mg kg<sup>-1</sup>), BaCl<sub>2</sub> – 0.45 (mean between 0.10 and 1.68 mg kg<sup>-1</sup>) and NH<sub>4</sub>OAc – 0.60 (mean between 0.17 and 2.43 mg kg<sup>-1</sup>). The mean values of hot water extractable B for alkaline soils were 1.87 (mean between 0.71 and 4.79 mg kg<sup>-1</sup>), CaCl<sub>2</sub> – 1.57 (mean between 0.45 and 5.43 mg kg<sup>-1</sup>), BaCl<sub>2</sub> – 1.37 (mean between 0.52 and 4.15 mg kg<sup>-1</sup>) and NH<sub>4</sub>OAc – 1.92 (mean between 0.85 and 8.33 mg kg<sup>-1</sup>) in acidic and alkaline soils respectively. The coefficient of variation for extractable B varied from 53.0 to 66.6 and 42.7 to 55.7 in acidic and alkaline soils respectively. The variation in B extracting efficiency in both the types of soils was

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found in the order: Hot water > Hot CaCl<sub>2</sub> > BaCl<sub>2</sub> > NH<sub>4</sub>OAc. Authors conclude that amongst all the compared extractants, 0.01 M CaCl<sub>2</sub> extraction may be an adequate procedure for B determination in both the acidic as well as alkaline soils.

*Keywords: Hot water-soluble boron; pH; Boron extractants; efficiency.*

## 1. INTRODUCTION

Boron is considered as one of the essential micronutrient which plays a crucial role in cell wall formation, pollen germination and pollen tube growth, imparting drought tolerance and helps in movement of sugars or energy into growing parts of the plants, ultimately its toxicity or deficiency may strongly affect the plant development. In general, boron exists in soils as in the form of water-soluble, absorbed, organically bound, and fixed in clay and mineral lattices. Of these forms, water-soluble B (readily soluble) has the greatest agricultural significance due its direct role in plant nutrition. Boron is the second most widespread micronutrient deficiency problem after zinc in Indian soils [1]. Because of the low B concentrations encountered in soil samples, its estimation in the laboratory is considered as a crucial step. Several methods have been used for determining Boron (B) concentrations in soils over the years. However, soil properties such as pH, texture, organic matter, and mineralogy have been found to be directly influence on determination of B. Also, B may be complexed in soils due to transformations through one or more of its several oxidative states. Therefore, there is a need to identify a suitable, precise and promising method for routine laboratory analyses of soil B.

Among various methods, hot water extraction of B is commonly used for the extraction of plant available B in Indian soils [2,3] despite having problem for colorimetric estimation of B due to organic matter and turbidity from suspended fine clay particles obtained during boiling of soil in water. Many researchers conducted the trials with suitable extractants for B in different soils. Studies conducted by Datta et al. [4] demonstrated that the hot calcium chloride was the most suitable for determining available B in acid soils of India followed by hot water, salicylic acid and ammonium acetate. However, salicylic acid appeared to be useful for routine analysis particularly for large number of samples. Sakal et al. 1993, while evaluating seven extractants for B in calcareous soils of North Bihar, found 1N NH<sub>4</sub>OAc (pH 7.0) as the promising extractant followed by hot water and 1N NH<sub>4</sub>OAc (pH 4.8) for chickpea crop[23]. Niaz et al. 2011 revealed

in their study that the 0.05 M HCl extraction method may substitute the hot water extraction method for plant-available B in alkaline and calcareous but moderately fertile soils [22]. For some acid soils of West Bengal, hot 0.01M CaCl<sub>2</sub> was found to be suitable extractant for assessing available B [5]. According to them, the suitability order was: hot CaCl<sub>2</sub> >Potassium di-hydrogen phosphate >Tartaric acid > CaCl<sub>2</sub>.mannitol. Jena et al. 2020 concluded that either salicylic acid or hot CaCl<sub>2</sub> method is suitable for boron extraction in red and lateritic soils of Odisha [20]. Considering the background information and research from the past, a prudent experiment conducted with two objectives as for comparison of different extractants and determining its suitability order for estimating available boron and finding promising extractant suitable for both acidic and alkaline soils.

## 2. MATERIALS AND METHODS

Representative sampling points were selected in the areas of Odisha and Andhra Pradesh which represents acidic and alkaline soils respectively. Two hundred surface soil samples (100 nos. from each site) at a depth of 15 cms were collected randomly from paddy-growing fields, using a stainless-steel soil auger. The latitude, longitude and elevation at each sampling site were recorded using a hand held global positioning system (GPS). Soil samples were air-dried, debris removed and sieved (2-mm) before analysis. For organic carbon analysis, soil samples were passed through 0.25 mm sieve. Determination of soil properties like soil pH and electrical conductivity (EC) were done on 1:2 soil: water (w/v) suspension using pH meter [6] and EC meter [7] following half an hour equilibration and organic carbon was determined by Walkley and Black - wet digestion method [8].

### 2.1 Determination of Boron by using four Different Extractants

In the present study, four extractants (Table 1) were used to determine extractable boron in selected 200 soil samples and the same procedure was followed for all extractants. Approximately, 10 g of soil sample was weighed and refluxed with 20 ml extractant (1:2 soil:

**Table 1. Different extraction methods used for the determination of boron**

| Method                                   | Extractant                                | Reference               |
|--|---|-------------------------|
| Hot water extractable method             | Hot distilled water                       | Berger and Troug, 1939  |
| Hot CaCl <sub>2</sub> extractable method | 0.01 M Calcium Chloride                   | Keren, 1996 [21]        |
| BaCl <sub>2</sub> extractable method     | 0.01 M Barium Chloride                    | De Abreu et al., [19]   |
| Ammonium acetate (pH-7.0)                | 1N Ammonium acetate (NH <sub>4</sub> OAc) | Sakal et al., 1993 [23] |

solution) by heating the sample for a period of 15 min at 150°C on block digester (Foss Analytics). Whatman no. 42 filter paper was used in filtration. An aliquot from the filtered extract was used for measuring B at wavelength 249.772 nm using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP OES - Teledyne Leeman labs). All the analyses were done by following standard protocols and keeping proper blanks and internal quality control checks and the results reported are mean of three replications (three readings of each sample).

In statistical analysis, simple correlation coefficients between the amounts of boron extracted by different extractants and other

chemical properties were worked out. In addition, Pearson's correlation was calculated by using windows based SPSS software.

### 3. RESULTS AND DISCUSSION

#### 3.1 Interaction of amount of Boron Extracted by Different Extractants with other Chemical Properties of Soil Samples

After thorough processing, the soil samples were analysed for selected chemical properties by following standard operating procedures and the results are presented in table 2a and 2b.

**Table 2a. Chemical properties of alkaline soils grouped according to soil pH with their range and mean values**

| No of samples       | pH (1:2)          | EC (dSm <sup>-1</sup> ) | OC (%)              | Boron (mg kg <sup>-1</sup> ) |                           |                           |                          |
|---------------------|-------------------|-------------------------|---------------------|------------------------------|---------------------------|---------------------------|--------------------------|
|                     |                   |                         |                     | Hot water extract            | CaCl <sub>2</sub> extract | BaCl <sub>2</sub> extract | NH <sub>4</sub> OAc pH-7 |
| 4                   | 6.5-7.0<br>(6.75) | 0.79-1.56<br>(1.27)     | 0.93-1.87<br>(1.32) | 2.35-3.16<br>(2.61)          | 1.80-2.22<br>(2.06)       | 1.49-2.30<br>(1.82)       | 1.84-2.44<br>(2.11)      |
| 21                  | 7.0-7.5<br>(7.29) | 0.14-2.06<br>(0.99)     | 0.59-2.03<br>(1.15) | 1.34-2.89<br>(1.98)          | 1.24-2.41<br>(1.61)       | 0.98-2.65<br>(1.46)       | 0.92-2.81<br>(1.50)      |
| 48                  | 7.5-8.0<br>(7.74) | 0.15-3.58<br>(0.95)     | 0.39-1.44<br>(0.88) | 0.76-4.79<br>(1.86)          | 0.50-3.88<br>(1.54)       | 0.52-3.44<br>(1.34)       | 0.85-4.84<br>(1.94)      |
| 23                  | 8.0-8.5<br>(8.21) | 0.14-2.27<br>(0.54)     | 0.22-1.23<br>(0.59) | 0.71-3.63<br>(1.72)          | 0.45-5.43<br>(1.54)       | 0.54-4.15<br>(1.30)       | 0.86-8.33<br>(2.22)      |
| 4                   | 8.5-9.0<br>(8.71) | 0.14-0.55<br>(0.32)     | 0.12-0.90<br>(0.53) | 0.79-2.04<br>(1.54)          | 0.56-1.88<br>(1.41)       | 0.56-1.92<br>(1.30)       | 0.98-2.52<br>(1.94)      |
| Total count (n) 100 | 6.6-8.9<br>(7.75) | 0.14-3.58<br>(0.85)     | 0.12-2.03<br>(0.88) | 0.71-4.79<br>(1.87)          | 0.45-5.43<br>(1.57)       | 0.52-4.15<br>(1.37)       | 0.85-8.33<br>(1.92)      |

**Table 2b. Chemical properties of acidic soils grouped according to soil pH with their range and mean values**

| No of samples       | pH (1:2)          | EC (dSm <sup>-1</sup> ) | OC (%)              | Boron (mg kg <sup>-1</sup> ) |                           |                           |                          |
|---------------------|-------------------|-------------------------|---------------------|------------------------------|---------------------------|---------------------------|--------------------------|
|                     |                   |                         |                     | Hot water extract            | CaCl <sub>2</sub> extract | BaCl <sub>2</sub> extract | NH <sub>4</sub> OAc pH-7 |
| 7                   | 4.0-4.5<br>(4.39) | 0.13-1.86<br>(0.84)     | 0.36-0.99<br>(0.60) | 0.27-0.37<br>(0.31)          | 0.18-0.49<br>(0.27)       | 0.15-0.86<br>(0.37)       | 0.19-0.52<br>(0.38)      |
| 39                  | 4.5-5.0<br>(4.73) | 0.04-1.74<br>(0.31)     | 0.34-1.05<br>(0.62) | 0.18-0.69<br>(0.34)          | 0.14-0.53<br>(0.34)       | 0.10-1.01<br>(0.37)       | 0.17-3.41<br>(0.59)      |
| 22                  | 5.0-5.5<br>(5.30) | 0.07-0.64<br>(0.18)     | 0.51-1.06<br>(0.82) | 0.29-1.03<br>(0.54)          | 0.25-0.71<br>(0.41)       | 0.21-0.68<br>(0.41)       | 0.23-1.10<br>(0.55)      |
| 17                  | 5.5-6.0<br>(5.69) | 0.03-0.43<br>(0.21)     | 0.10-1.00<br>(0.61) | 0.18-1.02<br>(0.68)          | 0.14-0.86<br>(0.54)       | 0.10-0.75<br>(0.52)       | 0.20-2.43<br>(0.90)      |
| 15                  | 6.0-7.0<br>(6.48) | 0.09-1.51<br>(0.35)     | 0.14-2.59<br>(0.79) | 0.31-1.50<br>(0.64)          | 0.29-1.52<br>(0.58)       | 0.24-1.68<br>(0.60)       | 0.34-1.61<br>(0.91)      |
| Total count (n) 100 | 4.3-7.0<br>(5.27) | 0.03-1.86<br>(0.32)     | 0.10-2.59<br>(0.68) | 0.18-1.50<br>(0.48)          | 0.14-1.52<br>(0.42)       | 0.10-1.68<br>(0.44)       | 0.17-3.41<br>(0.67)      |

**Table 3. Pearson correlation coefficients (r) between extractable boron content by different methods and soil properties**

| Extractant                 | Alkaline soils |         |                | Acidic soils |        |                |
|----------------------------|----------------|---------|----------------|--------------|--------|----------------|
|                            | pH             | EC      | Organic carbon | pH           | EC     | Organic carbon |
| Hot water (HWE)            | -0.246*        | 0.626** | 0.389**        | 0.516**      | 0.248* | 0.295**        |
| CaCl <sub>2</sub>          | -0.121         | 0.574** | 0.369**        | 0.459**      | 0.192  | 0.337**        |
| BaCl <sub>2</sub>          | -0.146         | 0.480** | 0.390**        | 0.203**      | 0.194  | 0.310**        |
| NH <sub>4</sub> OAc (pH-7) | 0.170          | 0.341** | 0.215**        | 0.265*       | 0.131  | 0.039          |

\*and \*\* denotes significant at 0.01 and 0.05 level respectively

The pH of the alkaline soils varied from 6.65 to 8.94 with a mean value of 7.75 whereas the pH of the acidic to neutral soils varied from 4.33 to 6.93 with the mean value of 5.27. Most of the alkaline soils (30%) shows soluble salts more than one indicating their moderately saline nature whereas the acidic soils consists low soluble salts within normal range. The organic carbon content of alkaline and acidic soils was ranged from 0.12 to 2.03% (0.88) and 0.10 to 2.59% (0.68) respectively. The available boron extracted by different extractants was found to be correlated with pH, electrical conductivity and organic carbon for acidic soils (Table 3). This reveals that mainly pH and organic carbon are in close association with the level of available boron present in soils [9,10,5]. Debnath and Ghosh [11] was also reported the positive correlations between soil pH and boron content in soils. Similarly, in alkaline soils, EC and organic carbon

were significantly positively correlated with all extractants but exceptionally, pH showing negative correlation with the extractants ( $r = -0.246^*$  with Hot water,  $r = -0.121$  with CaCl<sub>2</sub> and  $r = -0.146$  with BaCl<sub>2</sub>). Similar findings have been reported by Behera *et. al.*, 2016 [18]. Soil pH is one of the most important factor affecting boron levels as availability of B decreases with increasing soil pH, especially soil pH > 6.5. Levels of B adsorption by soil displayed close correlation with the pH of the soil solution has been strongly observed in several studies [12,13]

### 3.2 Available Boron Content Extracted by Different Extractants in Alkaline and Acidic Soils

Data regarding to the extractable boron by different extractants in both the soils has been presented in table 4. The ability of each

extractant to extract boron varies or depends on the bonding of elemental organic and inorganic compounds with the different B forms at various soil pH levels, this may ultimately intervene in the estimation of boron, its dynamics and extraction efficiency. During analysis, after filtration, an aliquot obtained from extractants except hot water was very clear, transparent and colorless extract.

Statistically, magnitude of extractability in the decreasing order of mean values and standard deviation was Hot water >CaCl<sub>2</sub> > BaCl<sub>2</sub> > NH<sub>4</sub>OAc in alkaline soils whereas Hot water >BaCl<sub>2</sub> >CaCl<sub>2</sub> > NH<sub>4</sub>OAc in acidic to neutral soils. As per the findings, among all extractants, the boron content was overestimated by ammonium acetate in both the soils. Cartwright et. al.[14] revealed that the use of ammonium acetate is considered to extract B by dissolving calcite surface in calcareous soils, which may account for the high levels of B removed by this extractant. Caballero et al., [15] evaluated available boron content using eight methods of extractions in different soils with pH between 4.1

and 8.2 from Cordoba and Sucre in Colombia, found that ammonium acetate had a higher extraction capacity than the other extractants.

### 3.3 Evaluation of Suitable Extractant in Determination of Available Boron in Alkaline and Acidic Soils

Correlation studies among the four extractants was computed to find the suitability of B extractants in acidic to neutral and alkaline soils. In general, the hot water extraction method is widely used as conventional for boron analysis and in the study, we considered it as a basic method to evaluate the other extraction methods. When analysing the contrasts between the different extractants in both the soils, the hot water extractant (HWE) was found to be significantly correlated CaCl<sub>2</sub> (r =0.869\*\* and r =888\*\*) and BaCl<sub>2</sub> (r =0.884\*\*and r = 730\*\*) followed by ammonium acetate (r = 0.701\*\* and r =390\*\*) in alkaline soils and acidic soils respectively (Table 5).

**Table 4. Statistical analysis of different B extractants in soil samples**

| Parameters         | Alkaline soils(100)     |                           |                           |                          | Acidic soils(100)       |                           |                           |                          |
|--------------------|-------------------------|---------------------------|---------------------------|--------------------------|-------------------------|---------------------------|---------------------------|--------------------------|
|                    | Hot water extract (HWE) | CaCl <sub>2</sub> extract | BaCl <sub>2</sub> extract | NH <sub>4</sub> OAc pH-7 | Hot water extract (HWE) | CaCl <sub>2</sub> extract | BaCl <sub>2</sub> extract | NH <sub>4</sub> OAc pH-7 |
| Range              | 0.71-4.79               | 0.45-5.43                 | 0.52-4.15                 | 0.85-8.33                | 0.18-1.50               | 0.14-1.52                 | 0.10-1.68                 | 0.17-2.43                |
| Mean               | 1.87                    | 1.57                      | 1.37                      | 1.92                     | 0.49                    | 0.42                      | 0.45                      | 0.60                     |
| Standard Error     | 0.08                    | 0.08                      | 0.06                      | 0.11                     | 0.03                    | 0.03                      | 0.03                      | 0.04                     |
| Standard Deviation | 0.80                    | 0.76                      | 0.63                      | 1.07                     | 0.26                    | 0.23                      | 0.27                      | 0.40                     |
| CV%                | 42.7                    | 48.4                      | 45.9                      | 55.7                     | 53.0                    | 54.7                      | 60.0                      | 66.6                     |

**Table 5. Mean contrasts of different extractants in both soils**

| Contrast                   | B available by methods<br>-----mg kg <sup>-1</sup> ----- |      | Difference | Significance |
|----------------------------|--|------|------------|--------------|
| <b>Alkaline soils</b>      |  |      |            |              |
| CaCl <sub>2</sub> vs HWE   | 1.57   | 1.87 | 0.30       | 0.869**      |
| BaCl <sub>2</sub> vs HWE   | 1.37   | 1.87 | 0.50       | 0.884**      |
| NH <sub>4</sub> OAc vs HWE | 1.92   | 1.87 | -0.05      | 0.701**      |
| <b>Acidic soils</b>        |  |      |            |              |
| CaCl <sub>2</sub> vs HWE   | 0.42   | 0.49 | 0.07       | 0.888**      |
| BaCl <sub>2</sub> vs HWE   | 0.45   | 0.49 | 0.04       | 0.730**      |
| NH <sub>4</sub> OAc vs HWE | 0.60   | 0.49 | -0.11      | 0.390**      |

\*\* denotes significant at 0.05 level respectively

The  $\text{CaCl}_2$  extraction showed positive association with the  $\text{BaCl}_2$  (0.954\*\*) indicates the feasibility of using  $\text{CaCl}_2$  as it is cheaper and non-toxic as compared to  $\text{BaCl}_2$  in the determination of boron in both the soils [16,17]. Moreover, barium chloride ( $\text{BaCl}_2$ ) extracts low concentrations of Boron as compared to other methods as it interferes in estimation of Boron on ICP-OES due to nearly similar wavelength lines. However, based on correlation studies and ease of estimation in colourless extract, relatively fast and economic, hot  $\text{CaCl}_2$  extraction method could easily replace conventional hot water extraction method for B estimation in acidic and alkaline soils.

#### 4. CONCLUSIONS

The findings of the study revealed all the extractants (0.01 M Calcium chloride ( $\text{CaCl}_2$ ), 1 N Ammonium acetate ( $\text{NH}_4\text{OAc}$ ) pH-7.0 and 0.01 M Barium chloride) that were tested for the determination of extractable B had positive correlation with soil pH, electrical conductivity and organic carbon of the experimental soils. On comparison with conventional hot water extraction method and other extractants chemically and statistically, it has been concluded that 0.01 M Calcium Chloride may be used as a suitable extractant for estimation of extractable boron in both acidic and alkaline soils.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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