Estimation of boron in soils, plants and water sample

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Boron occurs as anion in soils and is required by plants in very small quantity. Water soluble B makes the estimate of its availability to plants. Total boron in soils varies from 20 to 200 mg kg^{-1} and available (water soluble) boron in soils ranges from 0.03 to 12 mg kg^{-1}, respectively. The threshhold value ranging from 0.1 to 0.5 mg kg^{-1} (water soluble B) depends upon the soil type, crops, and other factors, below which the response to applied boron may be expected. Some sensitive crops to boron deficiency are listed in Table 1. Its availability is affected by soil pH as under:

– Deficiency of B is generally observed in old acid leached soils.

– Availability increased with the rise in soil pH having significant positive correlation with pH rising from 4.7 to 6.7.

– In neutral, saline and calcareous soils the B availability again decreases with the rise in soil pH having significant negative correlation with the rise in pH from 7.1 to 8.1. In calcareous soils B fixation occurs with the condensation of borate radical into long chains in the presence of Ca.

– In alkaline soils the availability of B is high and may be even toxic for plant growth.

– The low moisture availability also causes B deficiency.

Irrigation water containing Boron between 0.3 to 0.6 mg kg^{-1} can be used safely, whereas, irrigating soils with water containing 1 to 3 mg kg^{-1} B causes toxicity of B in plants.

Boron determination (Azomethine H Method):

Azomethine H forms coloured complex with H_3BO_3 in aqueous media. Over a concentration range of 0.5 to 10 µg B/ml the complex is stable at pH 5.1. Maximum absorbance occur at 420 nm with little or no interference from a wide variety of salts. This technique is rapid, reliable and more convenient to use than traditional procedures employing carmin, curcumin or quinalizarin (John *et al*., 1975).

Apparatus:

– Spectrophotometer

– Poly-propylene tubes 10 ml capacity.

Reagents:

– Distilled water

– Buffer solution: Dissolve 250 g of ammonium acetate (NH_4OAc) and 15 g of ethylenediaminetetracetic acid (EDTA disodium salt) in 400 ml of distilled water. Slowly add 125 ml of glacial acetic acid and mix.

– Azomethine H reagent: Dissolve 0.45 g of azomethine H in 100 ml of 1% L ascorbic acid solution. Fresh reagent should be prepared weekly and stored in a refrigerator.

Calcium hydroxide suspension: Add 0.4g $Ca(OH)_2$ to 100 ml distilled water.

– 0.1 N HCl : Add 8.3 ml conc. HCl to 900 ml distilled water, mix, cool to room temperature and make up the volume to 1000 ml.

– Calcium chloride 0.01 M Dissolve 1.11 g of anhydrous $CaCl_2$ in 900 ml distilled water and make up the volume to 1000 ml.

– Boron standard solution : Dissolve 0.114g of Boric acid (H_3BO_3) in distilled water and adjust the volume to 1000 ml. Each ml contains 20 µg B. Dilute 10, 20, 30, 40 and 50ml of the stock solution to 100 ml with distilled water to have solution with B concentration of 2,4,6,8 and 10 µg of B/ml, respectively. Include a distilled water sample for the 0.0 µg of B/ml standard solution.

Procedure :

Take 1 ml of aliquot of blank and diluted B standards into a 10 ml polypropylene tube, add 2 ml of buffer solution and mix. Add 2 ml of azomethine H reagent, mix and after 30 minutes read the absorbance at 420 nm on spetrophotometer. With the help of absorbance readings of standard solutions of different concentration of B the standard curve is drawn and a factor for concentration of B for 1 absorbance is calculated which is utilized to calculate B in the soils, plant or water sample.

Preparation of extracts :

Soil extracts : The hot water soluble extraction procedure of Berger and Truog (1939) is being used widely with slight modification of adding dilute electrolyte (0.01 M $CaCl_2$) instead of water only. This provides clear, colourless extract which eliminates the need of charcoal for decolourzation. Beside this a negative error, associated with B adsorption by charcoal, is also removed.

Place 20 g air dry soil in 250 ml low B flat bottom flasks and add 40 ml of 0.01 M $CaCl_2$ solution. Attach water cooled reflux condenser to the flask. Heat the flasks for 5 minutes and then cool and filter the suspension in plastic bottles.

Table 1 : Sensitivity of crop to boron deficiency

Highly sensitive	Medium sensitive	Low sensitive
Alfalfa	Apple	Barley
Cauliflower	Cabbage	Beans
Rape seed	Carrot	Corn
Conifers	Clover	Grasses
Peanuts	Cotton	Oat
Sugarbeet		Onion
Turnip		Pea
		Potato
		Soybean
		Wheat
		Rice

Transfer 20 ml aliquot to evaporating dish, add 2 ml $Ca(OH)_2$) suspension and evaporate the solution to dryness. Heat the evaporating dishes gently to destroy organic matter, cool to room temperature, add 5 ml 0.1N HCl. Triturate the residue with rubber policeman to ensure the complete dissolution of the residue (Bingham, 1982). For analysis of B pipette 1 ml of the aliquot and proceed as for the standard curve.

Plant digest sample: Take 0.5 g plant sample in porcelain/ platinum dishes Add 0.5 g $Ca(OH)_2$. Ignite the sample in the muffle furnace at 550°C for 4 hours to obtain white grey ash. Cool the dishes and moist the ash carefully with little distilled water and then add 5 ml 0.1N HCl. Transfer the content in to 25 ml volumetric flask mix and make up the volume to 25 ml with distilled water. For analysis of B take 1 ml of the aliquot and proceed as for the standard curve.

Water analysis sample: Take suitable quantity of water sample (containing 0.2 to 5.0 µg B) in porcelain dishes add 2 ml $Ca(OH)_2$ and proceed as described for soil extract. It is important to keep a definite volume of aliquot *i.e.* 1 ml of either soil, plant or water in final step of B determination.

References:

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