

cause formation and settling of the bed was not uniform, difficulty was encountered in having the same proportion of soil and fertilizer drawn in each core. This was indicated both by the P values and conductivity. These values were adjusted to the maximum conductivity found and this value was assumed to represent the best sample of the band. Since the Cu and Zn were less likely to diffuse from the band than the more mobile major cations, this correction applied to these elements was imperfect. By using the acid-extractable P which represented less mobility, the values for Cu and Zn were corrected to a constant P value and are also shown in Table IX. These values showed less fluctuations and indicated similar trends for release from the soil where organics and minor elements were applied as found using the conductivity correction. Obviously the organic-N increased the extractable Cu and Zn, but it was rather surprising to find all three bands alike in the degree of the release, since microbial activity was involved in the breakdown of the organics. These values for Cu and Zn were so much higher than in the unfertilized soil that it can be concluded without much doubt that the organics provided more available Cu and Zn for the crop.

The Cu and Zn released by the acid extraction of the soil were probably from chelated forms originating from residual organic-N material and reaction with such groupings in the soil organic matter. Reactions with clay colloids were considered negligible since less than 1%

clay was present. Acidity has previously been found to release Cu from soils in which much of the Cu was suspected of being in the chelated form (7, 4). These soil extracts, at pH 1.2 to 1.8, contained cupric ions, since dialysis removed the copper from solution and since this copper did not react with biquinoline, which is specific for cuprous ion, until reduction with hydroxylamine took place. As shown in Tables VII, VIII, and IX, where the organic-N sources had been treated with 3N HCl, part of the Cu and Zn was removed. However, the high buffer capacity of these organics prevented the acidity of leachates from falling below pH 4.5 in contrast to the soil extracts above. The colloidal leachates from AS, CP, and PG were dark in color, and direct extraction of these with biquinoline or dithizone reagents removed a very small proportion of the Cu and Zn in the leachates. This suggested that these metals were bonded more strongly to the organic matter present than in the reagent complexes with these metals. It was further confirmed that the metals were held in the organic matter, since neither Cu nor Zn was removed by dialysis. In the soil, the stability of Cu and Zn organic complexes from the organic-N sources has not been studied. For several years, the authors have attempted to study substitution of inorganic and chelated sources of Cu, Fe, and Mn or minor element mixtures for minor elements probably released from organic-N sources. The present studies have narrowed the problem concerning

which elements and what rates should be used, and indicated that sampling in the band and acid extraction of the soil samples should assist in obtaining correlation data in future experiments.

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SOIL BORON DETERMINATION

Modifications in the Curcumin Procedure for the Determination of Boron in Soil Extracts

THE most widely used method for determining microquantities of B in water extracts of soils is the curcumin procedure described by Dible *et al.* (2). While running many samples by this procedure, the author made several modifications in the method, thus allowing for greater reproducibility and accuracy.

Apparatus

Polypropylene beakers and centrifuge tubes.

Corning 728 glass reflux condensers and 100-ml. wide-mouthed extraction flasks.

Reagents

All reagents and standards are stored in polyethylene containers.

Flocculating Solution. Add slowly 1 gram each of Aerofloc No. 3171 and Superfloc No. 16 to ca. 400 ml. of hot, B-free H₂O, while agitating with mechanical stirrer. Continue heating and stirring until completely dissolved. Transfer to a 1-liter volumetric flask, cool, and bring to volume with H₂O. Both polyacrylamide polymers are presently known to be stocked by Fisher Scientific Co.

Extracting Solution. Dissolve 1 gram of reagent-grade CaCl₂·2H₂O in B-free H₂O in a 1-liter volumetric flask. Add

5 ml. of the flocculating solution and bring to volume.

Curcumin-Oxalic Acid Solution. To 400 mg. of curcumin and 50.0 grams of oxalic acid in a 1-liter volumetric flask add ca. 600 ml. of 95% ethanol. Heat gently with swirling until all reagents are dissolved. Cool, bring to volume with ethanol, and store in refrigerator.

Boron Standard. Dissolve 142.9 mg. of recrystallized H₃BO₃ in a liter of B-free H₂O. B concentration is 25 µg. per ml.

Dilute Boron Standard. Dilute 100 ml. of boron standard to 1 liter. B concentration is 2.5 µg. per ml.

Silicon Compensating Solution. Dissolve 1.265 grams NaSiO₃·9H₂O in a

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Clearer soil extracts were obtained by substitution of a 0.1% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ solution containing two polyacrylamide flocculating agents for distilled H_2O . Stability of the rosocyanine color was greatly improved by substitution of anhydrous acetone for 95% ethanol as the dye solvent. This substitution resulted in a shift in the absorbance maximum from 540 to 532 $m\mu$. Soluble silicate interfered seriously, especially at low concentrations of Si, when absorbance was measured at 532 $m\mu$ and a wide band utilized. This interference was obviated by using a Si compensating solution, a narrow band width at 532 $m\mu$, and/or measuring absorbance at 550 $m\mu$.

Table I. The Effect of Type of Extracting Procedure on the B and Si Concentration of the Extracts, $\mu\text{g. per ml.}$

Soil Type	Dible's Procedure (2)		Present Procedure	
	B	Si	B	Si
Cinebar loam	0.089	12.1	0.091	11.9
Alderwood sandy loam	0.136	11.9	0.136	11.9
Sultan silt loam	0.195	27.0	0.190	27.0
Puget silt loam	0.473	23.8	0.477	25.9
Felida silt loam	0.304	...	0.320	...
Puyallup sandy loam	0.260	...	0.260	...
Puget No. 1	0.515	...	0.550	...
Puget No. 2	1.950	...	2.020	...
Puget No. 3	1.300	...	1.200	...
Puget No. 4	0.725	...	0.700	...
Mean	0.595	18.7	0.594	19.2

liter of B-free H_2O . Si concentration is 125 $\mu\text{g. per ml.}$

Boron Standards for Developing Standard Curve. To a series of 25-ml. volumetric flasks, add 2 ml. of the Si compensating solution, zero to 20 ml. of the dilute B standard, and bring to volume with B-free H_2O . The concentration range is between 0 and 2.0 $\mu\text{g. of B per ml.}$

Procedure

Place 10 grams of sieved, air-dried soil into a 100-ml. extraction flask. Add 20 ml. of extracting solution, attach reflux condenser, and place on hot plate. Reflux for 5 minutes after boiling begins. Cool the flask in a shallow pan of water immediately following removal from the hot place to prevent excessive concentration of the extract by evaporation. Decant ca. 6 ml. into a polypropylene conical centrifuge tube; stopper, and centrifuge for 5 minutes at $900 \times G$.

Transfer 1 ml. of standards and unknowns to 50-ml. polypropylene beakers. Add one drop of Si compensating solution to both standards and unknowns. Add 4 ml. of curcumin-oxalic acid reagent, swirl, and evaporate to dryness on a water bath maintained at $55^\circ \pm 2^\circ \text{C}$. Continue heating at this same temperature for ca. 1 hour after beakers appear dry. Dissolve residue in 25 ml. of acetone (99.5 + %). Transfer immediately to stoppered conical polypropylene centrifuge tubes. Centrifuge at $900 \times G$ for 5 minutes. Measure absorbance at 550 $m\mu$ on an instrument having a band width of 20 to 30 $m\mu$. With a narrower band width (2 $m\mu$), a

532- $m\mu$ wavelength can be utilized with some increase in sensitivity.

Most humid-region soils do not produce extracts with more than 0.6 $\mu\text{g. of B per ml.}$ (1.2 p.p.m. in the soil) unless they were recently fertilized with borate. Sensitivity is improved, therefore, if standards range between 0.0 and 0.6 $\mu\text{g. per ml.}$ and residue is dissolved in only 10 ml. of acetone. Other standards and volumes of acetone may be substituted for those mentioned, depending on B levels of extracts.

Results and Discussion

New polyethylene storage bottles do not appear to contaminate reagents with B. A standard (25 $\mu\text{g. per ml.}$) solution stored for 3 years produced the same absorbance as a newly prepared standard. Initially, the dilute standard solution and working standards should be equilibrated for a period of 2 weeks with new polyethylene storage bottles and the same storage bottle used with any given standard solution thereafter. Standard curves are much more reproducible when this procedure is followed. Polypropylene ware is unaffected by the reagents and temperatures to which they are subjected in this procedure. Moreover, the problem of creeping does not exist during the drying step as it does when glass or ceramic ware are used. The cost of polyolefins compares very favorably to glass and ceramic ware and especially to B-free Corning 728 glass-ware. Although polypropylene pipets are on the market, they have not been satisfactory in this laboratory. No B contamination has been noted with the

use of soft glass volumetric equipment, although solutions are not stored in contact with this equipment for extended periods. After use, all laboratory ware other than storage vessels are soaked in a 0.05N HCl solution for at least 5 minutes previous to rinsing with B-free distilled H_2O . B-free H_2O is collected in a polyethylene carboy directly from a metallic still.

Extracting Solution. Dible *et al.* (2) require extraction of soil B by refluxing for 5 minutes with distilled H_2O , followed by the addition of a small amount of CaCl_2 to help clarify the extract. Jeffries and Thomas (3) found that use of two polyacrylamide flocculating agents aid in clarification of soil extracts for routine soil testing. In this laboratory, these materials did not perform well in distilled H_2O but were effective in a 0.1% solution of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$. To determine the most efficient concentration of the two polyacrylamides, various concentrations of each, both alone and in combination, were refluxed with an Alderwood sandy loam. The latter is particularly difficult to clarify. Absorbance at 545 $m\mu$ (Bausch and Lomb spectronic 20) was used as the criterion of efficiency. The materials were most efficient when used together. Concentrations of 0.1 mg. of each polymer per 20 ml. of 0.1% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ produced the clearest extract. Because higher concentrations increased colloidal dispersion, these polymers may also act as protective colloids.

Table I indicates that no consistent or significant differences were obtained between B or Si concentrations of samples extracted by the procedure recommended by the author and that recommended by Dible *et al.* (2). The original reason for adding CaCl_2 to the refluxed mixture was to flocculate the soil colloids (2). Because the present procedure is more efficient in this respect, it was adopted. It is also faster and more convenient, since transferring solid, deliquescent $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ to hot solutions presents obvious problems. The concentration of dissolved Si in soil extracts was determined by the procedure of Boltz and Mellon (7). It will be shown below that Si interferes with the determinations of B. Standard B solutions made up in distilled H_2O and the extracting solution recommended by

the author produced the same absorbance upon color development.

Curcumin-Oxalic Acid Solution. According to the procedure recommended by Dible *et al.* (2), the curcumin-oxalic acid solution should be made up daily. Williams and Vlavis (7) found this reagent remained stable when stored at 0° C. for 1 year. Similarly, this reagent remained stable for 3 months when stored in a refrigerator at 5° C. by the author. Longer periods of storage have not been attempted. It has been the practice to remove enough reagent from the refrigerator to do a day's work. The reagent is allowed to reach room temperature before use. Gentle heating to dissolve the curcumin more readily has not impaired its effectiveness.

Stability of Rosocyanine Solution. The red boron-curcumin complex (rosocyanine) fades quite rapidly after solution of the complex in 95% ethanol. Dible *et al.* (2) recommend this solvent for the dissolution of the dried rosocyanine residue previous to photometric determination of absorbance. Absorbance should be measured no later than 2 hours after color development. Rosocyanine is probably formed by a loose combination of H₃BO₃ and one of the hydroxyl groups of the curcumin molecule (2). Fading in 95% ethanol is probably due to hydrolysis.

When 99.5% acetone was used as the solvent for rosocyanine, no fading could be detected in solutions stored at room temperature up to 3 weeks. Longer storage was not attempted. Maximum absorbance occurs at 532 m μ for rosocyanine in acetone (Figure 1). The

absorbance peak in 95% ethanol is 540 m μ (2). Williams and Vlavis (7) studied means of improving the stability of rosocyanine both dry and in cold ethanol solution. Solution in anhydrous acetone, however, offers a much simpler answer to the problem.

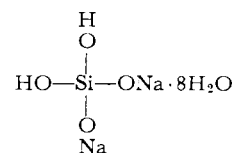
Silica Interference. Halvorson (4),

Table II. The Concentration of Soluble Si in Extracts of Various Soils Obtained by the Procedure Recommended for Soil B Determinations

Soil Type	Horizon	pH	Si, μg. per ml.
Mossyrock silt loam	A ₁	5.40	15.5
	A ₃	5.55	15.0
	AC	5.75	15.5
	C	5.70	15.0
Enumclaw sandy loam	A ₁	4.70	24.5
	B ₁	5.21	24.0
	B _{2g}	5.70	26.0
Chehalis silty clay loam	Ap	5.48	22.5
	B ₁₁	5.65	20.5
	B ₂₂	5.77	31.0
	B ₃	5.76	27.5
Nisqually sand	A ₁₁	5.48	31.0
	A ₁₂	5.65	13.5
	AC	5.86	15.5
Sultan silt loam	A	5.52	27.0
	AC	6.04	27.5
Greenwater sand	A ₁	6.15	7.0
	A ₂	6.15	2.5
	B _{21(ir)}	6.25	23.5
	B ₂₂	6.4	26.0
	C	6.20	15.0
Kopiah silty clay loam	Ap	5.00	12.0
	B ₁	4.90	16.0
	B _{21g}	4.70	35.0
	B _{22g}	5.05	43.5

using the Dible *et al.* (2) procedure, noted that soil extracts had higher apparent B concentrations when absorbance was determined at 540 m μ than at 554 m μ wavelength. This observation was confirmed by the author. In an attempt to determine the cause of this discrepancy, spectral analyses were made of the boron-curcumin complex produced with standard B solutions and soil extracts. As illustrated in Figure 1, the curve produced with a soil extract is distorted due to greater absorbance at the shorter wavelengths.

To determine the cause of this distortion, the B-curcumin complex was developed with standard B solutions which were contaminated with various ions at concentrations which might be expected to occur in the soil extracts. Ions investigated were NO₃⁻¹, Cl⁻¹, SO₄⁻², PO₄⁻³, SiO₄⁻⁴, NH₄⁺¹, Fe⁺², Fe⁺³, and Al⁺³. The only ion which produced the same distortion as the soil extract was SiO₄⁻⁴ (Figure 1). In this paper, Na₂SiO₃·9H₂O is referred to as the orthosilicate because McKeague and Cline (6) referred to it as such after a solution of the salt was passed through a H-resin. If they are correct, the structural formula might well be written as follows:



Their work (5) also indicates that soluble silicate in soil H₂O extracts is in a

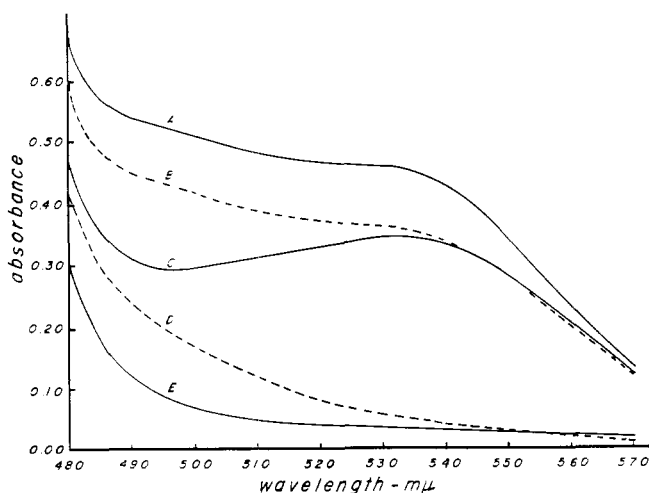


Figure 1. Spectral characteristics of curcumin complexes formed with and without B and Si and with a Puget soil extract

Color developed according to procedure outlined; 10 ml. of acetone were used to dissolve residue; Beckman DU spectrophotometer used to determine absorbance with a 0.02-mm. slit; theoretical band width is 1.2 m μ at the 550 m μ wavelength; (A) = Puget soil extract; (B) = 0.4 μg. B and 20 μg. Si per ml.; (C) = 0.4 μg. B and no Si per ml.; (D) = No B but 20 μg. Si per ml.; (E) = No B or Si.

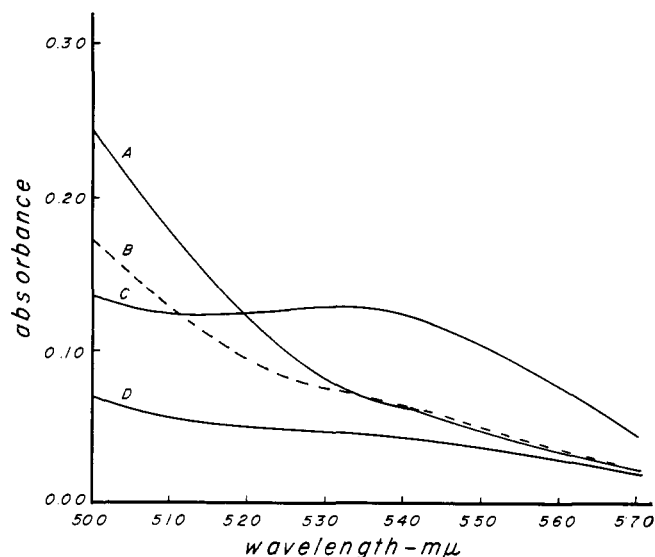
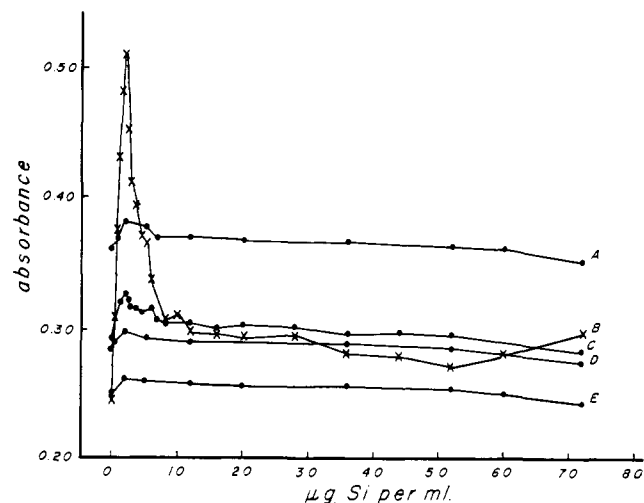


Figure 2. Spectral characteristics of complexes formed with curcumin and extracts of the Greenwater A₂ horizon containing 2.5 μg. of Si per ml. and the Kopiah B_{22g} horizon with 45 μg. Si per ml.

Color was developed according to procedure outlined in this paper, and residue dissolved in 10 ml. of acetone. (A) = Greenwater soil extract (B) = Kopiah soil extract; (C) = 0.1 μg. B per ml. standard; (D) = B-free distilled water

Figure 3. Spectral interference of various amounts of Si on the H_3BO_3 -curcumin complex formed with solutions containing a constant amount of B (0.4 $\mu\text{g. per ml.}$)

Color was developed according to the procedure outlined in this paper and residue dissolved in 10 ml. of acetone; absorbance determined on (A) Lumetron colorimeter with 550- $m\mu$ filter and 30- $m\mu$ band width; (B) Beckman DU spectrophotometer at 500 $m\mu$ with theoretical band width of less than 1.2 $m\mu$; (C) Beckman DU spectrophotometer at 532 $m\mu$ with theoretical band width of 1.2 $m\mu$; (D) Bausch and Lomb Spectronic 20 at 550 $m\mu$ with 20- $m\mu$ band width; (E) Beckman DU spectrophotometer at 545 $m\mu$ with theoretical band width of 1.2 $m\mu$



monomeric form which is probably weakly ionized $Si(OH)_4$.

As indicated in Figure 2, there is more interference from Si with an extract of the Greenwater soil than the Kopiah, even though the former contained much less Si than the latter (Table II). This anomaly is explained by the data illustrated in Figure 3. Absorbance rises sharply at the 500 $m\mu$ wavelength with increases in Si up to 2 $\mu\text{g. per ml.}$, and decreases sharply at higher levels of Si. When absorbance is measured at 532 $m\mu$ using a narrow band width, it remains quite constant between ca. 10 and 30 $\mu\text{g. of Si per ml.}$ This plateau is extended by moving to a higher wavelength with some sacrifice of sensitivity. Although not shown in Figure 3, the use of wider band widths, such as obtained with the Bausch and Lomb Spectronic 20 (20 $m\mu$), leads to more erratic results in the plateau area of Si interference when absorbance is measured at 532 $m\mu$. However, at 550 $m\mu$ very little change in absorbance is obtained between 10 and 50 $\mu\text{g. of Si.}$ At the higher levels of Si, absorbance is decreased at wavelengths of 532 $m\mu$ or longer. The reason for the differences in

sensitivity between instruments indicated in Figure 3 is primarily due to differences in the length of the light path used.

Because of the above, B standard solutions are made up to contain 10 $\mu\text{g. of Si per ml.}$ and the drop of Si compensating solution increases the level of Si in standards and unknowns 6 to 7 $\mu\text{g. per ml.}$ This should bring the standards and most unknowns into the plateau region of Si interference. If unknowns are known to be within the Si interference plateau region, no Si compensating solution need be added. Up to 5 drops of B-free H_2O can be added to beakers prior to addition of the curcumin-oxalic acid reagent without affecting absorbance of the rosocyanine. Thus, if a drop of Si compensating solution were added to the standards and not to the unknowns high in Si, there should be no effect on the apparent B levels. There are only a few soils that produce soil extracts high enough in Si that the additional Si will cause a slight depression in absorbance, and still fewer that are so low in Si that the added Si will not bring the level up to the interference plateau area (Table II).

If Si interferences were not com-

pensated for, only a small amount of positive error (Figure 3) would be made with most soils if absorbance is determined at 550 $m\mu$ with a wide band width or at 545 $m\mu$ with a narrow band. A change of 0.010 absorbance equals only ca. 0.014 $\mu\text{g. of B per ml.}$ with the least sensitive curve (D.U. 545 $m\mu$). Only with soils of extremely low Si levels is there chance of larger positive errors.

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