TRACING THE MINERAL FROM THE SOIL TO THE PLANT TO THE ANIMAL BLOOD

Part I. Effect of Lime on the Mineral Composition of the Soil, of the Grass, and on the Crop Yield

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The land in pasture, fallow, and idle in Puerto Rico, is estimated by the 1940 Census of Agriculture to be around 776,103 "cuerdas," of which the amount of acid land dispersed in the humid area may be estimated to be about 69.3 per cent or 537,696 "cuerdas." (1 cuerda = 0.971 acre)

How the application of lime to these soils would influence the mineral composition of the soil and of the grass is not known. According to Beeson (1), fundamental studies are lacking of what changes take place in the soil when the fertilizers are supplied, and on what effect these changes will have on the plant.

This paper reports the effect that a calcium application to an acid soil has on the composition of calcium, phosphorus, magnesium, manganese, and iron of the soil, of the grass grown in this soil, and on the yield of this crop.

EXPERIMENTAL WORK

Eighteen plots, each with an area of four-tenths of an acre, were selected in a field of "Fajardo clay" at the Experiment Station Farm at Río Piedras. "Fajardo clay" is an acid red soil of the humid region, derived from old, high alluvial material and from outwash fans of adjacent shale hills. The relief is level or gently sloping.

Limestone was added on June 25, 1943 to half of the randomized plots at the rate found by the lime-requirement test reported by Riera (4). The amount of limestone applied varied from 8 to 10 tons per acre. The field was planted in the middle of July 1943 with a mixture of Para grass *Panicum purpurascens*, and Carib grass *Eriochloa polystachya*, the former known as "Malojillo" and the latter as "Malojilla". Para and Carib grasses comprise the most valuable pasture and soilage grasses in the lowlands of the northeastern part of Puerto Rico.

Five consecutive crops were harvested on the dates reported in table 1. These dates varied for each crop because the grasses were cut daily, in strips, to supply to the stable herd. The grass from each strip was weighed in the field. The third and fifth crops were fertilized with ammonium sulphate at the rate of 500 pounds per acre. From January 29 to September 15, 1945, grass from the third to fifth crops inclusive, was supplied

daily to fifteen female goats used in a supplementary experiment to find the effect of the chemical composition of this grass on their health.

METHODS OF ANALYSES

SOILS

Three composite samples of the soil were taken from each plot; the first in June 1943 previous to the lime application; the second and the third, in September 1944 and May 1945, fifteen and twenty-three months, respectively, after the lime application. Each soil sample was analyzed for pH and for exchangeable calcium, magnesium, manganese, and for available iron and phosphorus.

Exchangeable Calcium, Magnesium, Manganese. Exchangeable calcium, magnesium, and manganese, were run by Peech's (3) method as follows: Weigh 10 grams of air-dried soil and leach into a 400 ml. beaker with about 225 ml. of normal neutral ammonium acetate solution. Dry leachate carefully in a hot plate and destroy organic matter and ammonium salts, adding 5 ml. of fuming nitric acid and 1 ml. of concentrated sulphuric acid and warming until the reaction has subsided and the brown fumes are no longer given off. Cool and rinse. Evaporate to dryness at low heat and continue heating for about 10 minutes to dehydrate the salts. Place the beaker in an electric muffle at 150°–200°C. and heat to 380°C. and hold at this temperature for 10–15 minutes. Treat residue with 3 ml. of 1:1 hydrochloric acid to dissolve the oxides of manganese and iron. Evaporate to dryness on steam bath and continue heating for fifteen minutes to dehydrate silica. Dissolve the salt residue with 10 ml. of 0.1 normal nitric acid. The solution should be colorless and clear, except for a trace of silica, which is either allowed to settle out in the beaker or centrifuge if necessary in a 15 ml. centrifuge tube. The solution from the beaker is decanted into a 15 ml. test-tube. This is solution A.

Transfer 2 ml. aliquot of solution A, equivalent to 2 grams of soil, to a 15 ml. centrifuge tube for the determination of calcium and magnesium. Add 0.2 ml. of ferric chloride solution (1 ml. = 1 milligram Fe), 3 ml. distilled water, and 2 ml. of 10 per cent sodium acetate solution. Mix and add 1 ml. of 0.1 normal sodium hydroxide, and mix again. Place the centrifuge tube in a water bath kept at 95°C. Add 1 ml. of a saturated solution of bromine, and maintain water bath temperature for at least one hour to flocculate the manganese dioxide, and to expel the excess of bromine. Add 2 ml. of 25 per cent ammonium chloride solution and digest for about 15 minutes. Add a drop of methyl red; and if the color of the indicator persists, indicating complete expulsion of bromine, remove the

tube from the water bath, cool, add 0.6 normal ammonium hydroxide from a burette until the color of the solution changes from a slightly red to a deep yellow; add 2 drops in excess. In general, it usually requires 0.5 ml. of 0.6 normal ammonium hydroxide. Make up to a volume of 13 ml. with water and add 5 drops of water in excess to allow for evaporation. Mix with a stirring rod and digest in water bath at 80°C. for 5 minutes to flocculate the precipitate. Centrifuge *while hot*, for 10 minutes. Designate as solution B.

Calcium. Pipette 10 ml. of solution B. equivalent to 1.5385 grams of soil, without disturbing the manganese-iron-aluminum precipitate, into a 15 ml. centrifuge tube. This is done best by holding the tube in front of a mirror. Add 0.5 ml. of 0.5 normal hydrochloric acid and 0.9 ml. of water and place in a water bath at 70°C. Mix by spinning the tube, add 2 ml. of 3 per cent ammonium oxalate. Mix thoroughly again and digest for 30 minutes at 70°C. Remove the tube from the bath and let stand for 30 minutes. The volume of the solution at this point is 13.4 ml. The excess of 0.4 ml. evaporates and the final volume of the solution is 13 ml. Decant the clear supernatant liquid into a dry test tube and keep the test-tube inverted at an angle of 45 degrees for a few minutes. Save the liquid for the magnesium determination, (Solution C). The precipitate of calcium oxalate remains in the test-tube. Add to the precipitate, 5 ml. of 2 normal ammonium hydroxide solutions saturated with calcium oxalate, break up the precipitate with a stirring rod, wash the rod, and centrifuge for 15 minutes at 1700 r.p.m. Decant the solution, drain the tube, and discard the clear liquid. Wash again, and centrifuge, if necessary. Dissolve the precipitate with 5 ml. of ten per cent sulfuric acid solution. Heat to 70°C. in a water bath and titrate with a standard 0.025 normal potassium permanganate solution.

The amount of calcium in soil is calculated as follows:

$$\text{p.p.m. Ca in soil} = (\text{ml. } KMnO_4 \times 0.025 \times 0.02004 \times 1{,}000{,}000) \div 1.5385$$
$$= 326 \times \text{ml. } KMnO_4$$

Magnesium. Take 10 ml. of solution C, equivalent to 1.1835 grams of soil, into a 15 ml. centrifuge tube. Place the tube in a bath at 70°C., add 0.8 ml. of 2 per cent alcoholic solution of 8-hydroxyquinoline, mix immediately by stirring, and then add 0.4 ml. of concentrated ammonium hydroxide from a buret. Stir vigorously for 1 minute, or longer if the amount of magnesium is extremely small, until full turbidity develops. Wash the stirring rod with a few drops of water and replace the centrifuge tube in a water bath at 70°C. for 10 minutes to flocculate the precipitate. If a number of magnesium determinations are to be carried out simultaneously,

set the centrifuge tubes aside after precipitation, until the magnesium in the last tube has been precipitated, then replace the tubes in a bath at 70°C. for 10 minutes. After digestion for 10 minutes, cool by immersing the centrifuge tubes in a bath at about 25°C., and allow to stand for 45 minutes to assure complete precipitation of magnesium; then add 0.5 ml. of 95 per cent ethyl alcohol slowly down the sides of the centrifuge tube, rotating the tube at the same time in order to wash down the precipitate and to form a layer of alcohol on the surface of the solution. Centrifuge for 15 minutes at 1700 r.p.m. and by using gentle suction draw off 2 to 3 ml. of the clear liquid to remove the layer of alcohol. Decant carefully and discard the solution; wipe the mouth of the tube with filter paper, add 5 ml. of ammoniacal ammonium acetate (8 ml. concentrated ammonium hydroxide in 300 ml. of 0.7 normal ammonium acetate), wash solution down the sides of the tube, break up the precipitate with a stirring rod, and wash the rod into the tube; add 0.5 ml. of alcohol down the sides of the tube to prevent creeping of the precipitate, and centrifuge for 15 minutes at 1700 r.p.m. Draw off the layer of alcohol, decant, and repeat the washing once more as directed above. Dissolve the precipitate in 4 ml. of 0.5 N hydrochloric acid, dilute to 13 ml. with water, stopper, and mix. Transfer a 1 ml. aliquot, equivalent to 0.0910 gram of soil to a 50-ml. volumetric flask, and add about 35 ml. of water, 5 ml. of 20 per cent sodium carbonate, and 3 ml. of phenol reagent, mixing the contents after each addition. Place the flask in boiling water for 1 minute, remove from the bath, and cool after 15 minutes. Make to volume, mix, and read in the spectrophotometer. The phenol reagent was prepared as follows: To 750 ml. of water in a 2-liter flask add 100 grams of sodium tungstate ($Na_2WO_4 \cdot 2H_2O$), 20 grams of phosphomolybdic acid ($20\ MoO_3 \cdot 2H_3PO_4 \cdot 48H_2O$), and 50 ml. of 85 per cent phosphoric acid. Boil for 2 hours, cool, and dilute to 1 liter with distilled water.

The transmittance-concentration curve (figure 1) for magnesium was developed as follows: Dissolve 0.15 gram of magnesium sulphate ($MgSO_4 \cdot 7H_2O$) in 100 ml. of 10 per cent ammonium chloride solution, heat to 60–70°C., add 10 ml. of the 8-hydroxyquinoline reagent, and make the solution alkaline with 4 ml. of concentrated ammonium hydroxide. Digest for 10 minutes, collect the precipitate on a fritted glass crucible, wash with hot dilute ammonium hydroxide, and dry at 140°C. Dissolve 0.0643 gram of the dried precipitate in 20 ml. of 0.5 normal hydrochloric acid and dilute to 500 ml. One milliliter contains 0.01 milligram of magnesium. Take 50 ml. of this standard solution and dilute to 100 ml. One milliliter of this second standard contains 0.005 milligrams of magnesium. The following transmittances were obtained, in a Coleman spectropho-

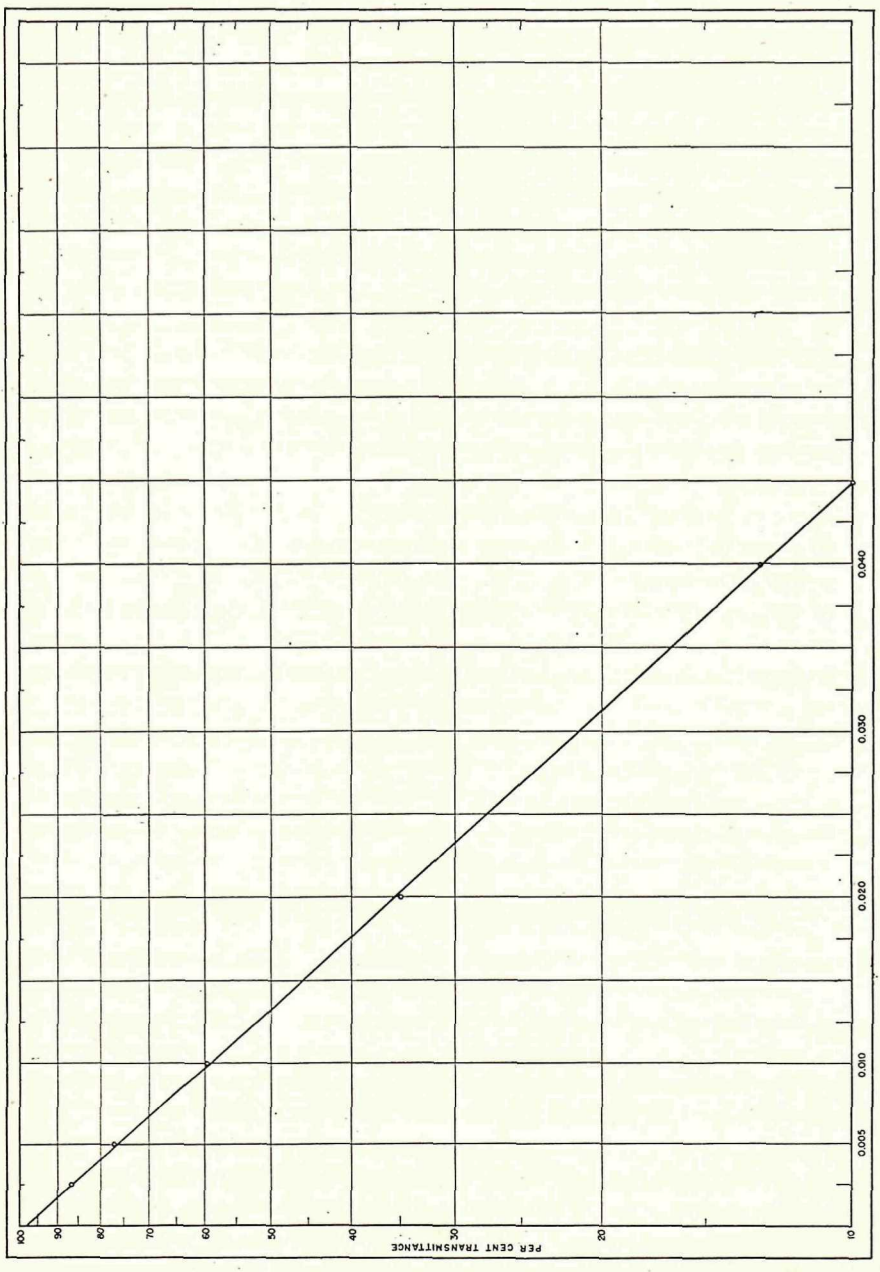

FIG. 1 Available magnesium in Soils and total magnesium in plants. Abscissa represents milligrams of magnesium as Mg.

tometer, model 11, using a PC-4 filter and a wave length of 650 mμ (figure 2), and a reagent blank as reference solution:

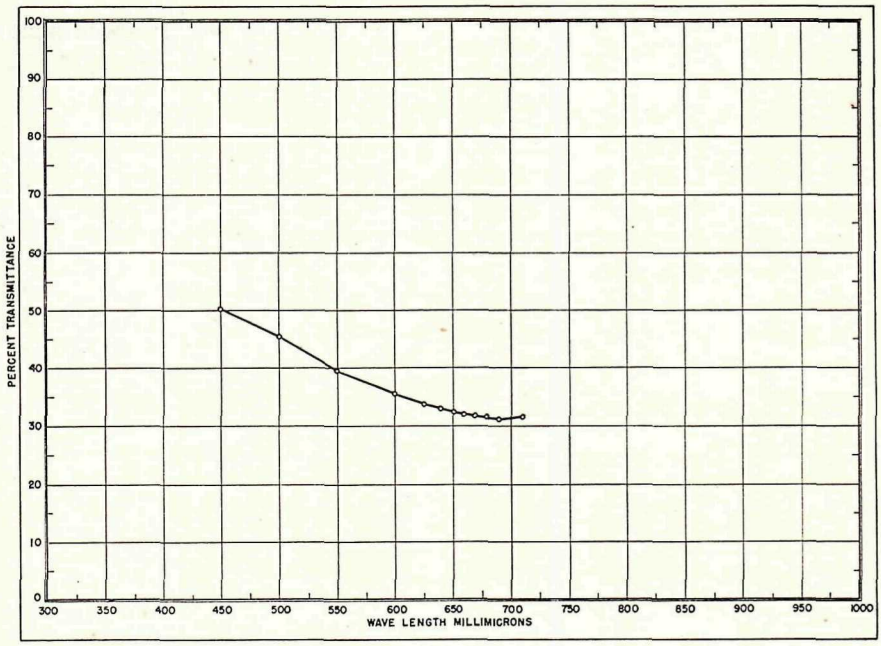

FIG. 2 Spectral-Transmittance curve for magnesium, as per method for soils and plants.

STANDARD MAGNESIUM SOLUTION		TRANSMITTANCE
ml.	*mg. Mg*	%
0.5	0.0025	87.0
1	0.005	77.2
2	0.010	59.8
4	0.020	35.1
8	0.040	12.9
10	0.050	8.9

The color was developed as explained in the procedure.

The amount of magnesium in soil is calculated as follows:

$$\text{p.p.m. Mg in soil} = \frac{\text{milligrams Mg in curve} \times 1{,}000{,}000}{1{,}000 \times 0.0910}$$

$$= \text{mgm. Mg} \times 10{,}989$$

Manganese. Manganese was determined by the simplified periodate method described by Peech (3). Transfer 2 ml. of solution, equivalent to 2 grams of soil, to a test-tube graduated at 11 ml. Add 1 ml. of 85 per cent

phosphoric acid, dilute to 11 ml. with water, and add 0.3 ml. to allow for evaporation, and mix with a stirring rod. Place in a water bath at 95°C., add about 50 milligrams of sodium periodate, mix thoroughly agair, and leave in the bath for 1 hour to assure full color development. Cool, make to volume if necessary; mix and read in the spectrophotometer.

The transmittance-concentration curve (figure 3) for manganese was developed as follows: To 22.8 ml. of 0.1 normal potassium permanganate solution in a 250 ml. Erlenmeyer flask, add about 50 ml. of water and a few drops of concentrated sulfuric acid. Heat to boiling and reduce the permanganate by the addition of sodium sulfite until the solution is colorless. Boil off the excess of sulfur dioxide and dilute to one liter. One milliliter of this solution is equivalent to 0.025 milligrams of Mn. The following transmittances were obtained in a Coleman spectrophotometer, model 11, using a PC-4 filter and a wave length of 525 mμ (figure 4) and a reagent blank as reference solution:

STANDARD MANGANESE SOLUTION		TRANSMITTANCE
ml.	*mg. Mn*	%
1	0.025	79.8
2	0.050	63.6
3	0.075	51.0
5	0.125	33.5
8	0.200	19.1
10	0.250	13.9

The color was developed as explained in the procedure.

The amount of manganese in soil is calculated as follows:

$$\text{p.p.m. Mn in soil} = \frac{\text{milligrams Mn in curve} \times 1{,}000{,}000}{1{,}000 \times 2}$$

$$= 500 \times \text{mg. Mn in curve}$$

Available Phosphorus and Iron. Available phosphorus and iron in the soil were extracted with Morgan's Universal extracting solution, normal sodium acetate buffered at pH 4.8 with acetic acid as follows: 12.5 grams of air-dried soil and 25 ml. of extracting solution were placed in a test-tube, 6″ long and 1″ in diameter. The tube was stoppered and shaken horizontally for 2 minutes, in a reciprocating shaker (Amer. Instrument Co. cat. ⁄7-155) at a speed of about 120 shaking cycles per minute. The extract was filtered in a Whatman filter paper No. 1.

Available Phosphorus—Phosphorus was precipitated as ammonium phosphomolybdate, reduced to the blue color with aminonaphtholsulfonic acid and determined colorimetrically as per Wolf's (5) procedure as follows: Take an aliquot of 5 ml. of soil extract, equivalent to 2.5 grams of soil,

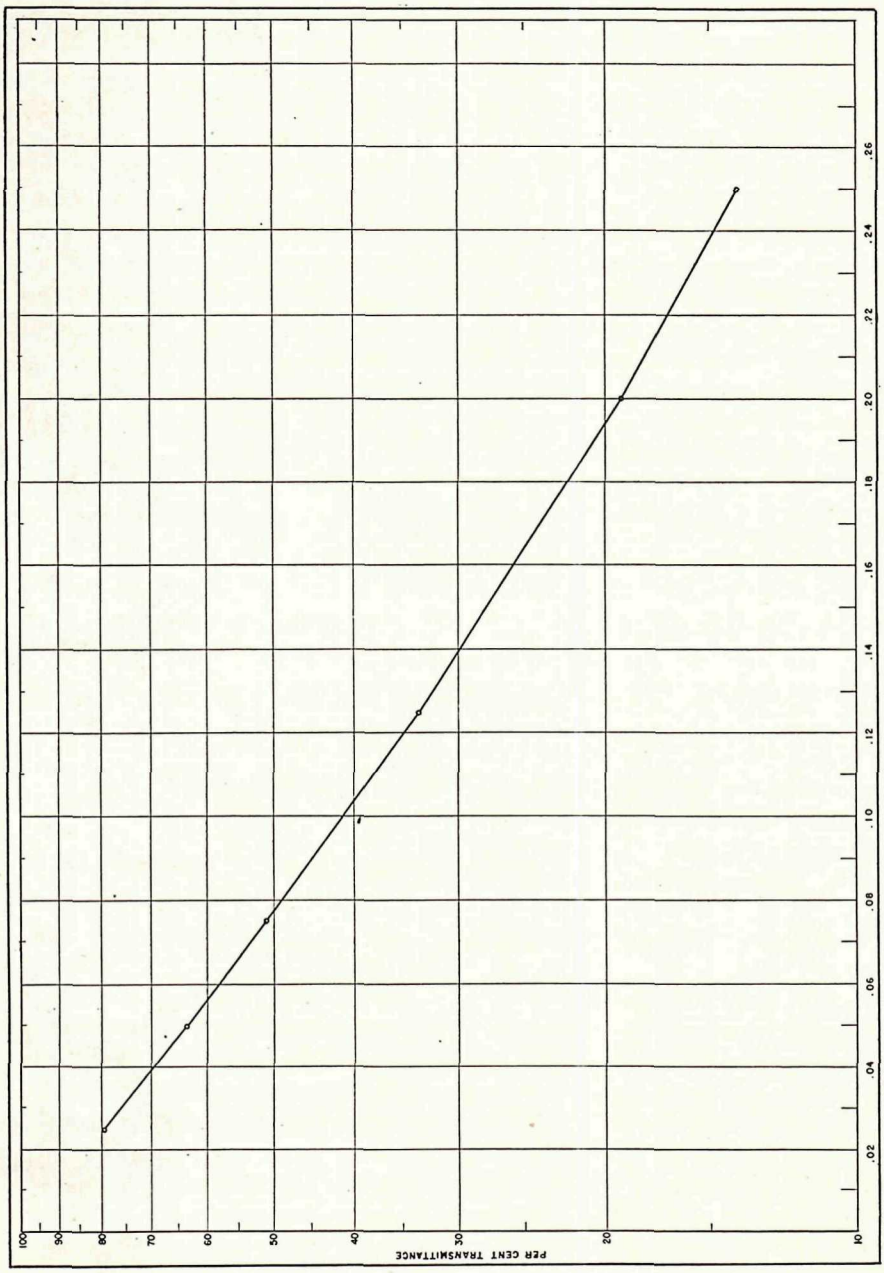

FIG. 3 Available manganese in soils and total manganese in plants. Abscissa represents milligrams of manganese as Mn.

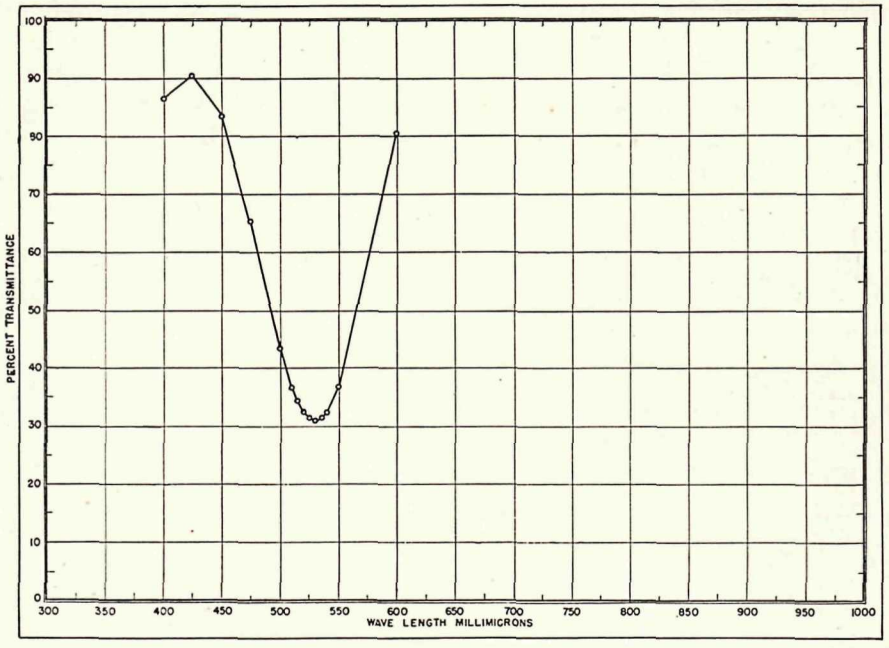

FIG. 4 Spectral-Transmittance curve for manganese as per method for soils and plants.

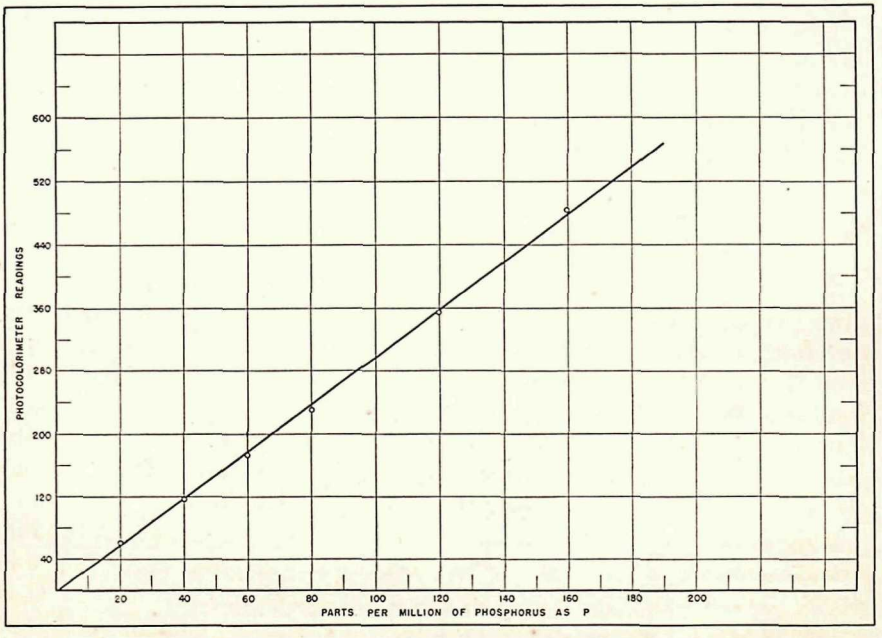

FIG. 5 Available phosphorus in soils.

dilute to 20 ml. with extracting solution, add 4 ml. of ammonium molybdate solution (2.5 per cent in 6 normal sulfuric acid, and 2 ml. of aminonaphthol-sulfonic acid solution (15 grams of anhydrous sodium bisulfite, are dissolved in 100 ml. of water, and 0.5 gram of pure, dry 1-amino-2-naphthol-4-sulfonic acid, and 1.5 grams of anhydrous sodium sulfite, are added; shake, make up to 500 ml. and store in a brown bottle).

The concentration curve for phosphorus (figure 5) was obtained in a Klett-Summerson photoelectric colorimeter No. 2141 with red filter 66 covering wave lengths from 640 to 700 mμ and instrument set at zero with reagent blank. The procedure was as follows: Weigh 0.1006 grams of sodium monobasic phosphate ($NaH_2PO_4 \cdot 2H_2O$) and dissolve in one liter of water. One milliliter of this solution is equivalent to 20 parts P per million. The following readings were obtained in the photoelectric colorimeter:

STANDARD PHOSPHORUS SOLUTION			PHOTOCOLORIMETER READING
ml.	*mg. P*	*p.p.m. P*	
1	0.02	20	60.5
2	0.04	40	115.4
3	0.06	60	174.4
4	0.08	80	229.9
6	0.12	120	353.5
8	0.16	160	483.0

The slope of this curve was found not to be constant. To check the slope, three phosphorus standards should be run with the unknown.

The amount of phosphorus in soil is calculated as follows:

$$\text{p.p.m. available P in soil} = \text{p.p.m. P in curve} \times \frac{1}{2.5}$$

$$= \text{p.p.m. P in curve} \times 0.4$$

Available Iron—An aliquot of 1 ml. of the soil extract equivalent to 0.5 gram of soil, was poured in a test-tube graduated at 10 ml. The color was developed as per method of Saywell and Cunningham, described by Parks et al (2), as follows: Add 1 ml. of 10 per cent hydroxylamine hydrochloride solution and 0.5 ml. of ortho-phenanthroline (1.5 per cent in 95 per cent ethanol), make to volume, mix and read in the photoelectric colorimeter. As the original extract was buffered to pH 4.8 there was no need of adjusting the pH with ammonium hydroxide as mentioned by Parks.

The concentration curve (figure 6) for iron was developed as follows: Weigh one-gram of c.p. iron wire in a liter volumetric flask and dissolve in about 150 ml. of 1:6 sulfuric acid; add 5 ml. of concentrated nitric acid as oxidizing agent; boil to expel SO_3 fumes, and complete volume to one liter.

One milliliter of this solution is equivalent to one milligram Fe. Ten milliliters of this solution were diluted to one liter; one milliliter of this solution contains 0.01 milligrams Fe. The following readings were obtained in a Klett-Summerson photoelectric colorimeter, No. 2141, with blue filter 42

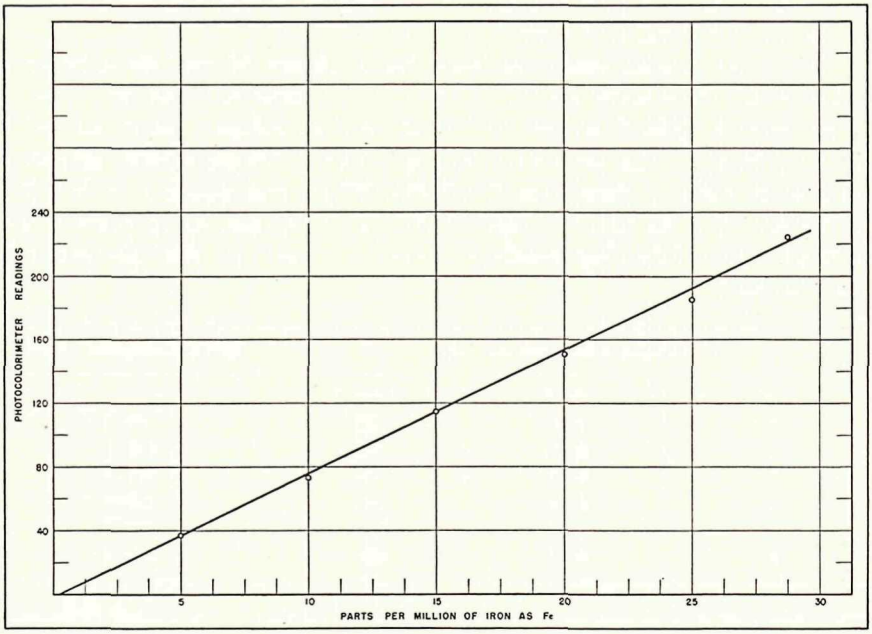

FIG. 6 Available iron in soils.

covering wave lengths from 400–465 mμ, and the colorimeter set at zero with reagent blank:

STANDARD IRON SOLUTION			PHOTOCOLORIMETER READING	SLOPE FACTOR
ml.	*mg. Fe*	*p.p.m. Fe*		
0.5	0.005	5	37	.1351
1.0	0.010	10	73	.1370
1.5	0.015	15	115	.1304
2.0	0.020	20	151	.1325
2.5	0.025	25	184	.1351
3.0	0.030	30	224	.1339

The color was developed as explained in the procedure. The slope of the curve was found to be constant; its average value is 0.1340.

p.p.m. available Fe in soil = p.p.m. Fe in curve × 2
= photocolorimeter reading × 0.1340 × 2
= 0.268 × photocolorimeter reading

GRASS

A composite sample (about ten pounds) of the standing grass was taken from each plot. The samples were dried to constant weight in a hot air oven at 150°F. Each sample was ground in a Wiley mill and sifted through a 1 mm. sieve. The ground samples were left overnight at room temperature to absorb atmospheric moisture. The dates of grass samplings are reported in table 1.

A 7.50 gram sample of dry grass was weighed in a 600 ml. pyrex beaker for each determination and the procedure of Parks et al (2), omitting the dithizone extraction, was followed as explained below.

Destruction of Organic Matter and Removal of Silica. Destroy the organic matter with nitric and perchloric acids; add first 12.5 ml. of concentrated nitric acid, place a cover glass on top of the beaker, and heat in a steam plate or hot plate at low temperature, in the hood; add again 12.5 ml. of nitric

TABLE 1

Dates of soil and grass samplings, of fertilizer application, of harvesting, and age of grass at harvest time

CROP	DATES OF SOIL SAMPLING	DATES OF GRASS SAMPLING	DATES OF AMMONIUM SULPHATE APPLICATION	HARVESTING DATES	AGE OF CROP HARVESTED	
					At start	At end
					mo.	*mo.*
First	6/43			1/13–27/44	5.5	6.0
Second	9/44	8/28/44		8/28–11/14/44	7.0	9.5
Third		11/16/44	10/10/44	11/15/44–3/29/45	2.5	4.5
Fourth	5/45	4/10/45		3/30–7/29/45	4.5	4.0
Fifth		7/16/45	4/25/45* 6/5/45*	7/30–10/25/45	4.0	3.0

* Only one application; dates refer to application for half of each plot.

acid and evaporate to near dryness. Add to residue 25 ml. of concentrated nitric acid and 25 ml. of 60 per cent perchloric acid. Do not add the perchloric acid before the nitric acid treatment because an explosion may occur. Evaporate to near dryness. Transfer residue quantitatively into a 125 ml. platinum dish, washing four or five times with 5 ml. portions of water. Add 5 to 8 ml. of 48 per cent hydrofluoric acid, from an 8 ml. beaker coated with paraffin, to the platinum dish; heat in hot plate carefully to dryness until silicon fluoride fumes are totally driven off. While working with grass samples from the dry area of Puerto Rico, a pink color persisted in this stage. It was destroyed by adding a pinch of peroxydisulfate ($K_2S_2O_8$) salt and a few drops of concentrated nitric acid. Cool, add 10 ml. of hot 0.6 normal hydrochloric acid, and dissolve the salts by continued heating and crushing of solid material with a flat end glass rod. Transfer to a 100 ml.

volumetric flask. Repeat heating-crushing operation, until the salts go in solution. Make up to 100 ml. volume with water and label, "Solution A"; 1 milliliter of this solution is equivalent to 0.075 gram of plant tissue.

Manganese. Manganese was determined by the simplified periodate method described by Peech (3). Pipette a 10 ml. aliquot of "Solution A" equivalent to 0.75 gram of plant tissue, into a 50 ml. beaker and evaporate to dryness, in a hot plate, to remove excess of hydrochloric acid. Dissolve residue in 6 ml. of normal nitric acid and transfer to a test-tube graduated at 11 ml. and follow the procedure explained before for the soils.

The transmittance-concentration curve (figure 3) was also developed as explained for the soils.

The amount of manganese in plant is calculated as follows:

$$\text{p.p.m. Mn in plant} = \frac{\text{milligrams Mn in curve} \times 1{,}000{,}000}{1000 \times 0.75}$$

$$= 1333 \times \text{mg. Mn in curve.}$$

Iron. Pipette 1 ml. of "Solution A" equivalent to 0.075 gram of plant tissue into a test-tube graduated to 10 ml. and develop color as explained before for soils.

Transmittance was measured this time in a Coleman spectrophotometer, model 11. The transmittance-concentration curve for iron (figure 7) was determined in the same standard used for soils. The following transmittances were obtained with filter PC-4, at a wave length of 490 mμ, using a reagent blank as reference solution:

STANDARD IRON SOLUTION		TRANSMITTANCES
ml.	*mg. Fe*	%
0.5	0.005	79.2
1.0	0.010	61.3
1.5	0.015	48.1
2.0	0.020	37.4
2.5	0.025	30.0
3.0	0.030	24.0

The amount of iron in plant is calculated as follows:

$$\text{p.p.m. Fe in plant} = \frac{\text{milligrams Fe in curve} \times 1{,}000{,}000}{1{,}000 \times 0.075}$$

$$= 13{,}333 \times \text{mg. Fe in curve.}$$

Phosphorus. Pipette a 0.1 ml. of solution A, equivalent to 0.0075 gram of plant tissue into a test-tube graduated at 10 ml. using a 0.1 ml.

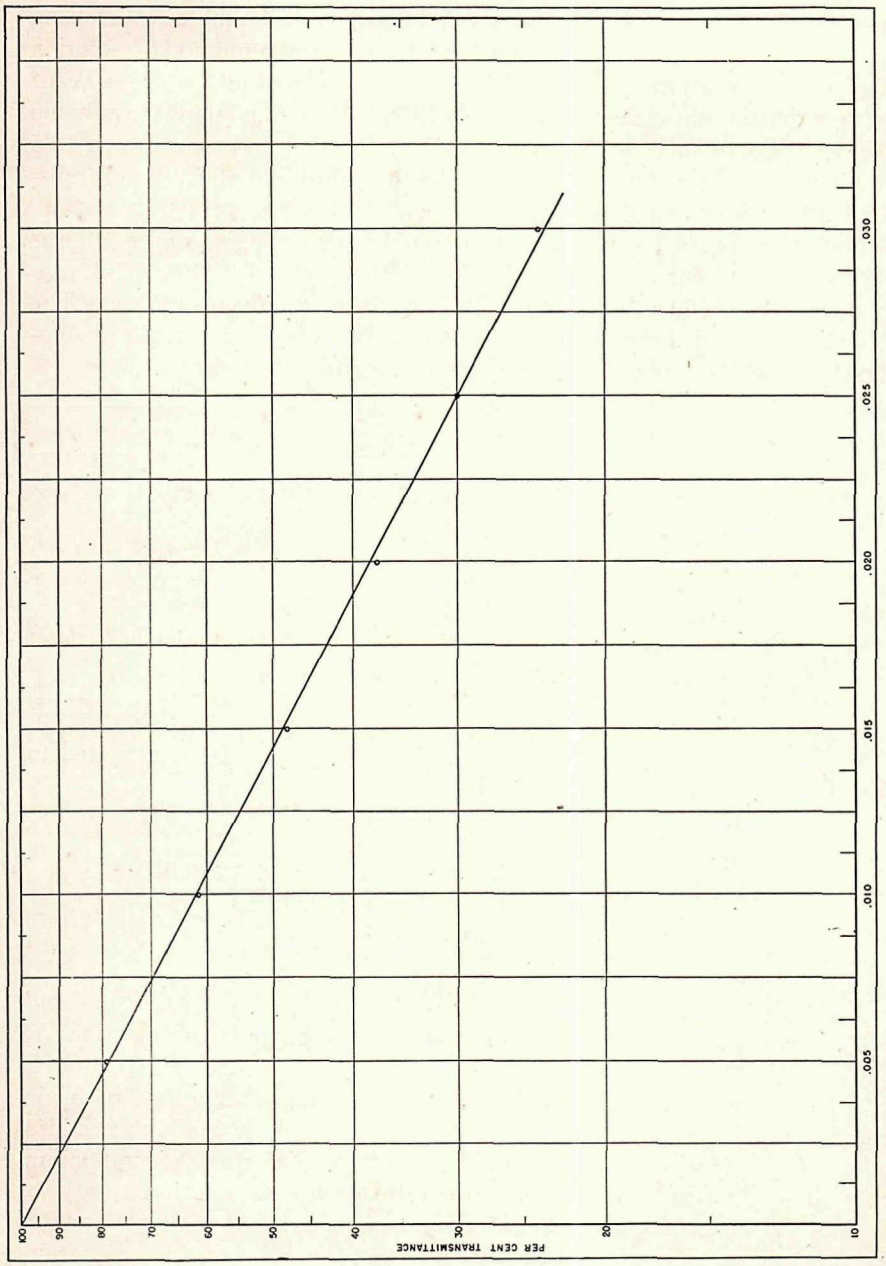

FIG. 7 Iron in plants. Abscissa represents milligrams of iron as Fe.

Mohr's pipette. Add 1 ml. of water and discharge the pipette by blowing with the tip under the water. Add 1 ml. of ammonium molybdate solution (2.5 per cent in 5 normal sulfuric acid), mix, and add 0.4 ml. of 0.25 per cent aminonaphtholsulfonic acid solution (0.125 gram of aminonaphtholsulfonic acid to 49 ml. of filtered 15 per cent sodium bisulfite, and then adding 1.25 ml. of 20 per cent sodium sulfite). Make to volume and mix. Read transmittance in spectrophotometer.

The transmittance-concentration curve for phosphorus (figure 8) was obtained in a Coleman spectrophotometer, model 11, with filter PC-4, at a wave length of 600 mμ, using distilled water as reference solution. The following transmittances were obtained in eight phosphorus standard solutions prepared as explained in the soils procedure:

STANDARD PHOSPHORUS SOLUTION		TRANSMITTANCE
ml.	*mg. P*	%
1.0	0.0050	83.1
1.5	0.0075	78.5
2.0	0.0100	72.9
3.0	0.0150	63.0
3.5	0.0175	58.6
4.0	0.0200	54.1
5.0	0.0250	46.7
5.5	0.0275	43.5

The slope of this curve was found not to be constant. It is suggested to run three standard solutions with the unknown.

The calculation of phosphorus in plant is as follows:

$$\text{p.p.m. P in Plant} = \frac{\text{milligrams P in curve} \times 1{,}000{,}000}{1{,}000 \times 0.0075}$$

$$= 133{,}333 \times \text{mg. P in curve.}$$

Removal of Iron, Aluminum, and Phosphorus Previous to Calcium and Magnesium Determinations. Transfer a 2.0 ml. aliquot of solution A, equivalent to 0.15 gram of plant tissue, to a 15 ml. centrifuge tube graduated at 13 ml. Add 0.2 ml. of ferric chloride solution (1.22 grams of ferric chloride hexahydrate in 250 ml. of 1 to 250 hydrochloric acid), mix, add 8 ml. of buffer solution (25 grams of sodium acetate, 62.5 grams of ammonium chloride, and 0.5 gram of sodium hydroxide in 1 liter of solution), and mix again. Add 1 drop of methyl red indicator solution (0.02 per cent) and 0.6 N ammonium hydroxide until the color of the solution changes from slightly red to deep yellow, and then add 2 drops in excess. Dilute to about 13.2 ml., mix with a stirring rod, and digest in a water bath at 80°C. for 5 minutes to flocculate the precipitate. Mix thoroughly, and centrifuge

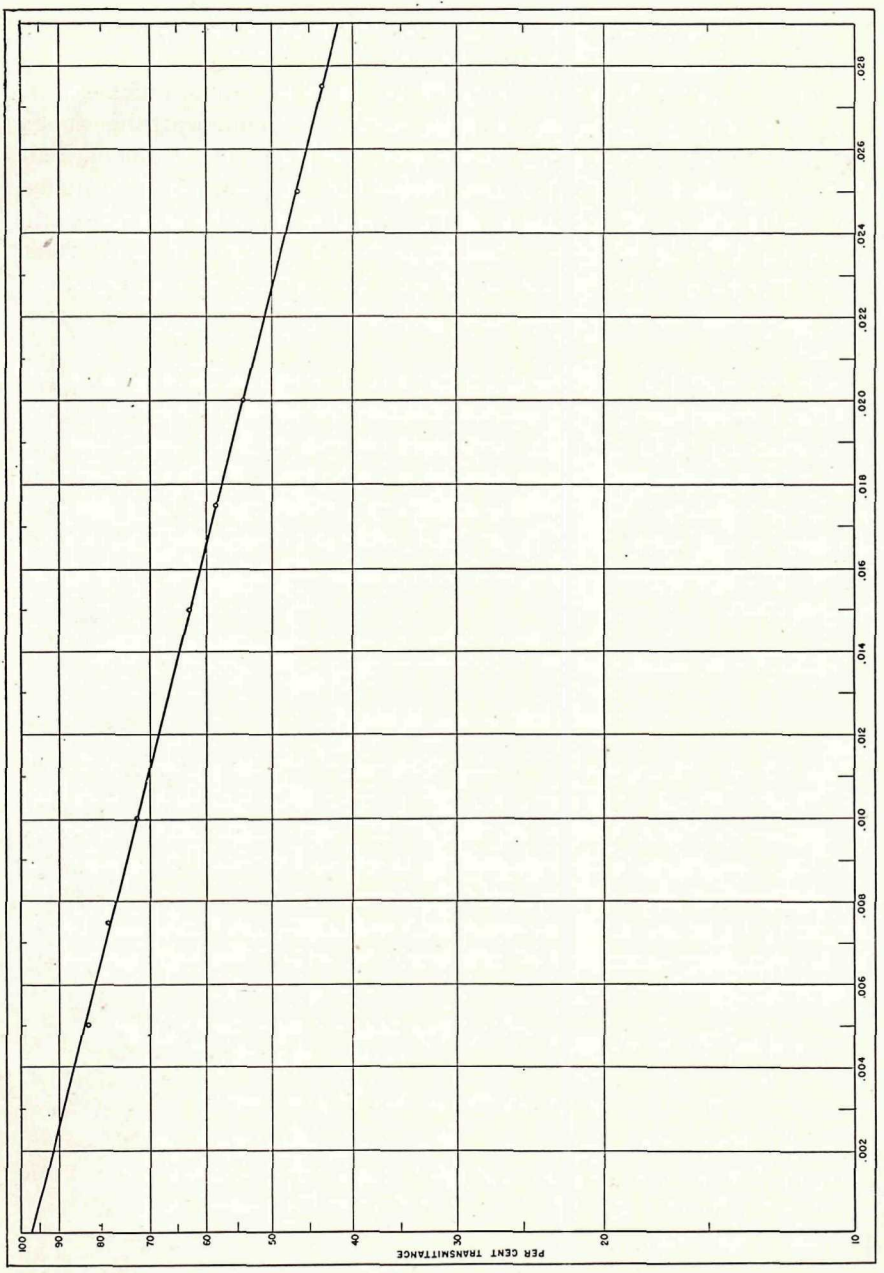

FIG. 8 Phosphorus in plants. Abscissa represents milligrams of phosphorus as P.

while hot for 10 minutes. The solution should have evaporated to 13.0 ml.

Calcium. Calcium was determined by the method of Peech (3). Transfer a 10 ml. aliquot from the above 13 ml. clear solution, equivalent to 0.1154 gram of plant tissue, into another calibrated 15 ml. centrifuge tube; add 1.4 ml. of 0.2 normal hydrochloric acid, and place in a water bath at 70°C. Mix; add 2 ml. of 3 per cent ammonium oxalate, mix thoroughly again, and digest for 30 minutes at 70°C. Remove the tube from the bath and let stand for 30 minutes. Centrifuge for 15 minutes, at about 2000 r.p.m. The volume should now be 13 ml. Decant the clear supernatant liquid gently into a 25 ml. test-tube and save for the magnesium determination.

Allow the centrifuge tube to drain for several minutes, inclined at a 45° angle, on a filter paper. Add quickly from a pipette, about 5 ml. of 2 normal ammonium hydroxide saturated with calcium oxalate, centrifuge for 15 minutes, decant carefully and discard the solution. Drain the tube and save the precipitate. One washing is sufficient unless very large quantities of calcium are present. Add about 5 ml. of 10 per cent sulfuric acid, heat to 70°C. on a water bath, and titrate with standard 0.025 normal potassium permanganate.

$$\text{p.p.m. Ca in plant} = \frac{\text{ml. } KMnO_4 \times 0.025 \times 0.02004 \times 1{,}000{,}000}{0.1154}$$

$$= 43{,}413 \times \text{ml. } KMnO_4$$

Magnesium. Magnesium was also determined by the method of Peech (3). Pipette 10 ml. of supernatant liquid, from the solution set aside for the magnesium determination, equivalent to 0.0887 gram of plant tissue, in a 15 ml. centrifuge tube graduated at 13 ml. and proceed as described in the magnesium determination reported before for soils. Take a 2 ml. aliquot from the 13 ml. solution, equivalent to 0.01365 gram of plant tissue, and develop color as mentioned previously for soils. Read transmittance in curve (figure 1).

$$\text{p.p.m. Mg in plant} = \frac{\text{milligrams Mg in Curve} \times 1{,}000{,}000}{1{,}000 \times 0.01365}$$

$$= 73{,}260 \times \text{mg. Mg in Curve}$$

Proteins, Ether Extract and Fiber. Proteins, ether extract, and fiber were determined in the first, second and third crops. Proteins were also determined in the fourth crop, previous to and after the second application of ammonium sulphate.

PRESENTATION AND DISCUSSION OF DATA OBTAINED

The mineral changes brought about in the soil, fifteen and twenty-three months after the lime application, are expressed in table 2.

The increase of available calcium and phosphorus and the decrease of available iron in the soil due to liming, was highly significant, fifteen and twenty-three months after the lime was applied to the soil. The decrease of available manganese in the soil due to liming was highly significant fifteen months after liming and significant twenty-three months after liming. The difference between the available magnesium content of the limed and unlimed soil was not significant.

TABLE 2

Parts per million of available calcium, magnesium, manganese, phosphorus, and iron in soil type Fajardo clay, unlimed and limed (dry basis)

TIME OF SAMPLING	TREATMENT	CALCIUM (Ca)	MAGNESIUM (Mg)	MANGANESE (Mn)	PHOSPHORUS (P)	IRON (Fe)
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
15 months after liming	Unlimed	849	180	42	13	17
	Limed	6831	172	8	61	2
23 months after liming	Unlimed	992	156	29	21	45
	Limed	5351	156	5	56	12

TABLE 3

Parts per million of calcium, magnesium, manganese, phosphorus and iron in three crops of Para-Carib grass grown in soil Type Fajardo Clay, unlimed and limed (air dry basis)

CROP NUMBER	TIME OF SAMPLING	TREATMENT	CALCIUM (Ca)	MAGNESIUM (Mg)	MANGANESE (Mn)	PHOSPHORUS (P)	IRON (Fe)
			p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Second	14 months after liming	Unlimed	2199	1509	229	2100	149
		Limed	2811	1638	137	2430	158
Third	17 months after liming	Unlimed	2008	1824	156	2749	196
		Limed	3351	2212	84	3047	160
Fourth	32 months after liming	Unlimed	2919	2166	243	2450	124
		Limed	3381	2088	181	2929	121

The mineral changes brought about in the grass after the lime application are expressed in table 3.

The increase of calcium and the decrease of manganese in the grass due to liming was highly significant for the second and third crops while the increase of calcium was significant for the fourth crop, and the decrease of manganese was not significant. The increase of phosphorus in the grass due to liming was highly significant for the second and fourth crops but was not significant for the third crop. There was no significant change in the iron

content of the grass due to liming in the three crops and in the magnesium content of the second and fourth crops. However, the increase of magnesium in the grass crop due to liming was highly significant for the third crop.

The average total yield of green grass per acre in the unlimed and limed soil for each of the first five consecutive crops, and for the five crops, is reported in table 4.

The increase in the grass yield due to liming was significant for the first and third crops. However, the difference between the respective yields of the unlimed and limed soil for the second, fourth and fifth crops, and for the total of five crops, was not significant.

TABLE 4

Yield in tons per acre of green Para-Carib grass in Fajardo clay unlimed and limed

TREATMENT	NUMBER OF CROP					TOTAL
	1 No nitrogen applied	2 No nitrogen applied	3 Nitrogen applied	4 No nitrogen applied	5 Nitrogen applied	
Unlimed	8.98	7.47	9.59	8.92	9.82	44.78
Limed	11.00	8.03	10.33	8.62	9.81	47.79

TABLE 5

Grass yields of table 4 expressed as tons per acre per month of green grass

TREATMENT	NUMBER OF CROPS AND AGE IN MONTHS				
	1 5.75 mo.	2 8.25 mo.	3 3.50 mo.	4 4.25 mo.	5 3.50 mo.
Unlimed	1.56	.91	2.74	2.10	2.81
Limed	1.91	.97	2.95	2.03	2.80

The monthly rate of growth for each of the five grass crops is reported in table 5. The age of the crop used for this calculation was the mean of that reported in table 1.

The increase in the yield of the third grass crop is due to nitrogen fertilization. It gave about two tons of green grass more per acre than the previous crop (table 4). The monthly rate of growth was about three times higher (table 5). In a period of 7.75 months, the third and fourth crops combined gave close to 5 tons of green grass per acre, while the unfertilized second crop in 8.25 months gave about one ton. In fourteen months the first two unfertilized crops gave about three tons of green grass per acre.

However, in about a period of one year the last three crops gave about eight tons of green grass per acre. The eight-ton year yield was obtained with two applications of nitrogen fertilizer, one to the third crop and another to the fifth crop, each at the rate of 500 pounds of ammonium sulphate per acre.

The increase of grass yield is not the only advantage obtained when nitrogen is applied. The content of the nitrogen in the crop is also increased if the grass is cut early (table 6).

The protein content of the Para-Carib grass mixture ranged between 3 and 4 per cent. Grass from the third crop taken 36 days after the first nitrogen application gave around 11 per cent protein or about three times that in the original grass. The ammoniacal content in the third crop was .07 and .05 per cent, respectively, for the unlimed and limed grass. The protein content of the fifth grass crop, collected 82 days after the nitrogen application, was about 5 per cent.

TABLE 6

Protein content of Para-Carib grass in five consecutive crops, before and after nitrogen fertilization (air-dried-basis)

TREATMENT	CROP 1, NO NITROGEN APPLIED	CROP 2, NO NITROGEN APPLIED	CROP 3, 36 DAYS AFTER FIRST NITROGEN APPLICATION	CROP 4, 180 DAYS AFTER FIRST NITROGEN APPLICATION	CROP 5, 82 DAYS AFTER SECOND NITROGEN APPLICATION
	%	%	%	%	%
Unlimed.....	3.6	3.8	11.7	3.3	4.8
Limed.....	3.8	3.7	10.8	3.6	4.5

SUMMARY

This paper reports the procedures followed for the chemical determinations of exchangeable calcium, magnesium, and manganese; and available phosphorus and iron in soils; and for the total amount in plants of each of those minerals mentioned. Spectrophotometric methods are given for magnesium and manganese in soils and plants; and for phosphorus and iron in plants including the transmittance-concentration and spectral-transmittance curves for each of these elements. Photocolorimetric methods are also given for available iron and phosphorus in soils with their corresponding curves.

This paper reports also changes of the minerals calcium, magnesium, manganese, phosphorus and iron in an acid soil, 15 and 23 months after liming. It also reports changes of these minerals in each of five crops of a mixture of Para grass *Panicum purpurascens*, and Carib grass *Eriochloa polystachya*, grown in the unlimed and limed soil. The yield of green grass is also reported for each crop.

The important results are as follows:

1. Significant increases of available calcium and phosphorus and significant decreases of available manganese and iron in the soil, due to liming, and no significant difference of the available magnesium content.
2. Significant increase of calcium and significant decrease of iron in each of three consecutive crops of the grass, due to liming. Significant decrease of manganese in the first two crops but no significant difference in the third crop. No significant difference in the magnesium content of the first and third crops but a significant difference in the middle crop.
3. The increase in the grass yield, due to liming, was significant for the first and third crops but was not significant for the second, fourth, and fifth crops, or for the total of the five consecutive crops.
4. An application of 500 pounds of ammonium sulphate per acre gave about two tons of green grass more per acre than a previous unfertilized crop. The period of growth of the fertilized grass was 3.5 months while that of the unfertilized grass was 8.25 months.
5. Grass collected early, 36 days after the nitrogen application, contained around 11 per cent of protein or about three times as much as in the unfertilized grass.

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