

carbon. However, it should be pointed out that the actual amount of carbon, although water-insoluble, was much greater than that applied in the wheat straw extract. On the basis of unit amount of carbon, the amount of total nitrogen at the end of 28 days was generally greater for the soluble source of carbon, the only exceptions being at the higher rates of nitrogen added. Jensen (12) found that hot-water extracted straw did not favor any nitrogen fixation; it appears that cold-water extracted straw retains some available carbon sources for *Clostridium* if not for *Azotobacter*. Possibly hot and cold water have different effects on the physical structure and availability of the extracted straw.

The results of laboratory experiments, conducted under optimum conditions, are difficult to compare with results likely to occur in the field where distribution of straw, fertilizer, and moisture are irregular and variable. Nevertheless trends are indicated and inferences may be drawn. There is no doubt that straw additions influence nitrogen balance in the soil by promoting assimilation of

available nitrogen, by affecting nitrification, by favoring denitrification, and by stimulating nitrogen fixation. Summation of these transformations determines the net loss or gain of available and total nitrogen.

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## SOIL MAGNESIUM ANALYSIS

# Elimination of Manganese Interference in the EDTA Titration of Exchangeable Soil Magnesium

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Manganese titrates quantitatively and interferes in the determination of magnesium by EDTA [disodium (ethylenedinitrilo)tetraacetate] titration. This interference can be conveniently eliminated by addition of ferrocyanide to precipitate the manganese. Exchangeable soil magnesium can be titrated by EDTA with Eriochrome Black T (EBT) as the indicator in the presence of 1 meq. of exchangeable manganese per 100 grams of soil. If larger amounts of manganese are present, the precipitate of manganese ferrocyanide obscures the end point and must be removed by filtration before magnesium is titrated.

THE REAGENT EDTA [disodium(ethylenedinitrilo)tetraacetate] is widely used in the determination of calcium and magnesium. Cheng and Bray (1) adopted the procedures originated by Schwarzenbach, Biedermann, and Bangerter (3) to determine calcium directly and magnesium indirectly in plant materials and soils. To determine magnesium directly, calcium must first be removed. Tucker and Kurtz (4) obtained the most consistent results by tungstate precipitation of calcium.

Titrations with EDTA are subject to interference by foreign ions, such as

aluminum, cobalt, copper, iron, manganese, and nickel, which are sometimes present in small but detectable amounts in soil and plant materials (1). Heavy metals such as cobalt, copper, nickel, and a small quantity of iron can be complexed with potassium cyanide (7). Pribil (2) screened aluminum from interference with triethanolamine, which also masked a small quantity of iron. When present in larger quantities, iron usually precipitates as the hydroxide at the pH specified for the titration.

Methods for eliminating manganese interference have not been convenient

or satisfactory. This paper proposes a convenient procedure for such elimination in the titration of magnesium with EDTA. In neutral and alkaline solutions, manganese ferrocyanide is insoluble, but the calcium and magnesium salts of ferrocyanide are highly soluble and ionized in solution. Ferrocyanide precipitation was very effective in removing manganese interference from the titration of calcium and magnesium with EDTA. This modification gave very reproducible results when used in the analysis of soil for exchangeable magnesium.

## Reagents

**STANDARD MAGNESIUM SOLUTION.** Magnesium sulfate heptahydrate (5.068 grams) dissolved in 1 liter of distilled water (500 p.p.m. magnesium solution).

**STANDARD CALCIUM SOLUTION.** "Special" low magnesium reagent grade calcium carbonate (2.497 grams) was dissolved in a minimum quantity of 1 to 1 hydrochloric acid and diluted to 1 liter with distilled water (1000 p.p.m. calcium solution).

**STANDARD MANGANESE SOLUTION.** Manganese sulfate monohydrate (1.54 grams) was dissolved in 1 liter of distilled water (500 p.p.m. manganese solution).

**STANDARD EDTA SOLUTION.** A stock solution of 0.2% disodium dihydrogen (ethylenedinitrilo)tetraacetate has a convenient concentration for soil analysis. This solution was standardized against both the calcium and the magnesium standards.

**EBT INDICATOR.** Eriochrome Black T (0.2 gram) was dissolved in 50 ml. of methanol containing 2 grams of hydroxylamine hydrochloride. Fresh indicator should be prepared every 3 weeks to assure a sharp end point.

**BUFFER SOLUTION (pH 10).** Ammonium chloride (67.5 grams) was dissolved in 200 ml. distilled water, mixed with 570 ml. of concentrated ammonium hydroxide and diluted to 1 liter.

**TUNGSTATE SOLUTION.** Twenty grams of sodium tungstate were dissolved in 100 ml. of distilled water.

**Interference-Removing Reagents.** Potassium Cyanide Solution. Two grams of potassium cyanide were dissolved in 100 ml. distilled water.

Triethanolamine, pure reagent grade.

Potassium Ferrocyanide. Four grams of reagent grade potassium ferrocyanide trihydrate were dissolved in 100 ml. of distilled water.

## Titration Procedures

**Determination of Calcium Plus Magnesium.** A suitable aliquot was diluted to about 100 ml. and adjusted to pH 10 by adding 15 ml. of ammonium hydroxide-ammonium chloride buffer. Ten drops of each of the interference-removing reagents and 10 drops of EBT indicator were added. Precipitation of manganese ferrocyanide was almost instantaneous when solutions were at 40° C. or warmer. When solutions were cold, titrations were delayed a few minutes to allow time for the precipitation to go to completion. Titrations were made from a microburet, with the standardized EDTA under fluorescent light, to the end point indicated by the color change of EBT from wine-red to pure blue.

**Determination of Magnesium.** A

**Table I. Effect of Ferrocyanide in Eliminating Manganese Interference**

Present, Mg.			Mg + Ca Found as Ml.-EDTA Equivalent	
Mg	Ca	Mn	Without Fe(CN) <sub>6</sub>	With Fe(CN) <sub>6</sub>
0.50	0	0	4.06	4.06
0.50	0.50	0	6.40	6.41
0	0	0.50	1.74	0
0.50	0	0.25	4.92	4.08
0.50	0	0.50	5.80	4.07
0.50	0	2.50	12.72	Interfered
0.50	0.50	0.50	8.10	6.42
0.50	Removed	0.50	5.84	4.10

**Table II. Precision of Determination of Exchangeable Calcium and Magnesium in Cisne Soil**

Sample	Aliquot	Ca + Mg as Ml.-EDTA Equivalent	Magnesium		Calcium	
			EDTA, ml.	Mg.	EDTA, ml.	Mg.
I	1	8.18	3.61	0.445	4.57	0.976
	2	8.16	3.63	0.447	4.53	0.968
	3	8.16	3.60	0.444	4.56	0.974
	4	8.17	3.58	0.441	4.59	0.980
II	1	8.15	3.56	0.439	4.59	0.980
	2	8.12	3.54	0.436	4.58	0.978
	3	8.15	3.53	0.435	4.62	0.987
	4	8.15	3.54	0.436	4.61	0.985
III	1	8.18	3.60	0.444	4.58	0.978
	2	8.15	3.57	0.440	4.58	0.978
	3	8.17	3.60	0.444	4.57	0.976
	4	8.14	3.58	0.441	4.56	0.974
IV	1	8.19	3.62	0.446	4.57	0.976
	2	8.14	3.58	0.441	4.56	0.974
	3	8.19	3.61	0.445	4.58	0.978
	4	8.18	3.60	0.444	4.58	0.978

suitable aliquot was diluted to about 100 ml. and 20 ml. each of ammonium hydroxide-ammonium chloride buffer and tungstate solution were added. This mixture was warmed for 1 hour, allowed to cool, filtered through No. 42 Whatman filter paper, and washed with a solution containing 50 ml. of buffer per liter. Ten drops of each of the interference-removing reagents were added to the filtrate, which was then titrated in the manner described for calcium plus magnesium.

## Results with Standard Solutions

Table I gives examples of data obtained by titrating solutions containing a constant amount of magnesium and different amounts of manganese with EDTA in both the presence and absence of ferrocyanide.

In the absence of ferrocyanide, manganese ions reduced the intensity of the metal-indicator complex so that more indicator was required for the detection of the end point. In other respects, manganese did not interfere with the end point, but titrated quantitatively along with the calcium and magnesium. Data in Table I show further that manganese, alone, in the absence of calcium and magnesium, was titrated quantitatively by EDTA with the EBT indicator

and that manganese interference was not affected by the removal of calcium by tungstate precipitation.

In the presence of ferrocyanide, manganese did not influence the titration when present in amounts less than 0.5 mg. If larger amounts of manganese were present, the precipitated manganese ferrocyanide interfered with end point detection, and should be filtered out before the titration is made. If magnesium alone were determined in the presence of large amounts of manganese, the ferrocyanide could presumably be added with the tungstate, so that calcium and manganese would be removed simultaneously. Potassium ferrocyanide at the concentration of 4% has only a light yellowish color that does not affect the detection of the color change of the EBT indicator.

## Results with Soil Extracts

Because consistent and reproducible results were obtained with standard solutions, the method was applied to soil analyses. Results obtained are illustrated in Table II with data from determinations on an acid Cisne soil (No. S 24065) from southern Illinois.

As the determinations of amounts of calcium and magnesium are of interest here, the exchangeable cations were removed from the soils by an equilibrium

**Table III. Statistical Analysis of Precision of Analytical Methods**

Analytical Method	Mean, $\bar{Y}$	Variance, $S^2$	Standard Deviation, $s$	Coefficient of Variation, %
Ca + Mg	8.161	0.000308	0.01755	0.22
Mg direct	0.4416	0.00000445	0.00211	0.48
Ca indirect	0.9776	0.0000130	0.00360	0.37

extraction rather than by an exhaustive leaching. Four samples of 20 grams of soil were weighed, soaked in 100 ml. of 1*N* neutral ammonium acetate solution for 1 hour, agitated on a shaker for 30 minutes, and filtered through Whatman No. 42 filter paper. Aliquots of 10 ml. of this extract, each representing 2 grams of soil, were suitable for either direct determination of magnesium or the combined calcium and magnesium determination by the titration procedures previously described. The calcium equivalent of EDTA was obtained by subtracting the magnesium equivalent of EDTA from that of calcium plus magnesium.

The precipitated manganese ferro-

cyanide was slight and caused no difficulty in titrations, although Cisne soil is relatively high in easily soluble manganese. With the weight of soil sample and size of aliquots used in these experiments, interference from exchangeable manganese in amounts less than 1 meq. per 100 grams of soil is eliminated simply by the addition of ferrocyanide. If the amounts of manganese are higher, the precipitate should be removed by filtration to avoid difficulty in detection of the end point.

Data in Table II are from four aliquots of extracts of each of the four samples of soil and show excellent agreement within samples as well as among samples.

Statistical analyses of these data are shown in Table III. Coefficients of

variation were 0.22, 0.48, and 0.37%, respectively, for the calcium plus magnesium titration, magnesium titration, and calcium determination by difference. Validity of these statistical statements is limited to the operator and conditions of these experiments, but good agreement is indicated.

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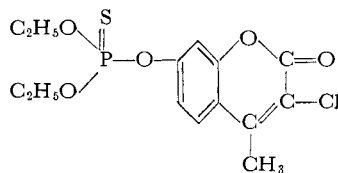
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## INSECTICIDE ASSAY

### Assay of Co-Ral in Technical Material and Formulated Products

Two analytical methods for the determination of Co-Ral in technical material and formulated products are described. These involve alkaline hydrolysis and ultraviolet absorption spectrophotometry. The former method gives the more precise results. However, the latter allows for easy correction for 3-chloro-4-methylumbelliferone in the material and is less time-consuming than the former. Both methods have been used successfully for routine control purposes.

CO-RAL, formerly known as Bayer 21/199, is a commercial insecticide for the control of various insect pests of domestic animals. The compound is *O,O*-diethyl-*O*-3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl phosphorothioate. The structural formula is



For the analysis of both technical material and formulations, a reproducible method of assay was required. The literature contains numerous references to analytical methods for coumarin derivatives, exemplified by the work of Ensminger (2). However, most of these methods have been designed for trace analysis and were not considered

sufficiently accurate for assay purposes.

A preliminary examination of possible methods of analysis of the above compound indicated alkaline hydrolysis and ultraviolet absorption to be the most promising techniques for further study. Both of these approaches have been investigated and both have yielded satisfactory analytical procedures.

From a consideration of the structure of Co-Ral, hydrolysis was expected to form diethylphosphorothioic acid from saponification of the ester grouping and hydrochloric acid from the chlorine in the lactone ring. Ring opening in alkaline solution should form a carboxylic acid which should also be titratable. In addition to this, the above reactions would form two weakly acid phenolic groups. If ring closure to form a benzopyran ring occurs (9), only the remaining phenolic group would be titratable. Thus one might expect to

obtain four or possibly five equivalents of acid on alkaline hydrolysis of Co-Ral.

The aromatic structure of Co-Ral suggests the use of ultraviolet absorption as an assay method. Coumarin derivatives (2) are known to be strong absorbers in the ultraviolet. In addition to this, Hensel *et al.* (5) have described an ultraviolet absorption method for the unchlorinated derivative of Co-Ral (Potasan).

A fluorescence method for determination of Co-Ral residues in animal tissues has been published recently (7).

#### Experimental

**Hydrolysis of Co-Ral.** The alkaline hydrolysis of Co-Ral was investigated in both water and isopropyl alcohol. Samples of recrystallized Co-Ral were hydrolyzed by refluxing for 2 hours in 1*N* aqueous and 1*N* isopropyl alcoholic

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