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Simple modification of the Walkley-Black method for simultaneous determination of organic carbon and potentially mineralizable nitrogen in tropical rice soils

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Key words Acid dichromate Anaerobic incubation Release of ammonium Total N.

Summary A modified version of the Walkley – Black method of organic C determination is described, which allows simultaneous determination of organic C and potentially mineralizable N in soils. As usual the organic matter of the soil is oxidized by acid dichromate reagent by heat of dilution. The excess of chromate left after C oxidation is titrated with standard ferrous sulfate solution instead of ferrous ammonium sulfate to avoid contamination due to NH_4^+. The NH_4^+ released due to oxidative action of acid dichromate is determined by distilling a suitable aliquot from the known volume with 50% of NaOH solution. The ammonium released by this method with 15 surface soils showed highly positive correlations with organic C (r = 0.969**), total N (r = 0.976**) and the NH_4^+ produced under waterlogged conditions (r = 0.985**). The modification is very simple and is easily adaptable for estimation of potentially mineralizable N in soils alongwith organic C determination.

Introduction

Our previous findings showed that the organic carbon content determined by the Walkley-Black[8] method was highly correlated with the potentially mineralizable N (NH_4^+) released under waterlogged conditions in a large number of diverse Philippine wetland rice soils[3,4]. Further, it was found that the acid dichromate extractable NH_4^+ was a good index of soil nitrogen availability to wetland rice in a greenhouse pot study with 39 diverse soils having a wide range in pH, organic matter and texture[6]. Taking leads from these studies[5,6], Sahrawat[6] suggested that the ammonium released by oxidative action of acid dichromate during organic carbon determination offered a good opportunity to determine both organic C and potentially mineralizable N in soils simultaneously on the same sample. The work reported here provides results to show that a simple modification of the Walkley-Black method described in this paper can be conveniently adapted to estimate organic C and potentially mineralizable N simultaneously on the same sample. Study with 15 surface samples showed that the NH_4^+ released by the oxidative action of acid dichromate was highly correlated with organic C, total N and mineralizable N released under waterlogged conditions.

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Materials and methods

Soils

The soils used (Table 1) were surface samples (0–15 cm) of Philippine soils selected to have a wide range in pH (4.5–7.9), organic C (0.63–5.46%), total N (0.06–0.60%) and clay content (22–68%). The soils samples were collected from important rice growing areas of the Philippines[6]. The samples were air-dried and crushed to pass through a 2-mm sieve for use in this study. Soil analyses reported in Table 1 was done as described by Sahrawat[6].

Simultaneous determination of organic C and mineralizable N

The Walkley-Black method as described by Jackson[2] was adapted with simple modifications to determine in the same sample both organic C and mineralizable N. The 0.5 g soil sample was placed in a 500-ml conical flask, 10 ml of 0.17 *M* $K_2Cr_2O_7$ was added through a pipette. The soil and dichromate were mixed by gently swirling the flask, followed by addition of 20 ml of concentrated H_2SO_4. The flask was again swirled gently to allow soil to have good contact with the reagent. The contents of the flask were allowed to stand for 30 minutes, followed by dilution with 200 ml of water, 10 ml of 85% H_3PO_4, 0.2 g of NaF and 20–25 drops of diphenylamine indicator. The contents were titrated with 0.5 M ferrous sulfate solution (Dissolve 140 g of reagent grade $FeSO_4.7H_2O$ in water, add 15 ml of conc. H_2SO_4 and make the volume to 1000 ml). The final volume was made to a known volume. The flask was shaken vigorously to mix the contents and allowed to stand for 5–10 minutes. A 20 ml or suitable aliquot from the flask was distilled with 50% aqueous solution of NaOH. The ammonia distilled was absorbed in boric acid-indicator mixture and titrated with 0.4 *M* H_2SO_4 to determine the amount of NH_4^+ released[1]. A standard blank without soil was run and titrated similarly.

As noted under introduction back titration of the excess chromic acid is carried out with standard ferrous sulfate solution rather than with ferrous ammonium sulfate to avoid contamination due to NH_4^+ because ammonium released by acid dichromate oxidation is to be estimated.

Table 1. Analyses of soil used

Soil		pH	Organic C	Total N	Clay
No.	Texture	(1 : 1 H_2O)	(%)	(%)	(%)
1	Silt loam	7.9	0.63	0.06	23
2	Silty loam	7.5	0.63	0.06	23
3	Silty clay loam	7.2	0.84	0.07	41
4	Silty clay loam	7.0	0.91	0.08	44
5	Silty clay	5.7	1.03	0.09	48
6	Clay	5.6	1.15	0.10	68
7	Silty clay loam	5.3	1.36	0.11	32
8	Clay	4.5	1.54	0.17	62
9	Silty clay loam	6.4	1.76	0.18	33
10	Clay loam	7.4	1.97	0.18	33
11	Clay	6.6	2.14	0.21	68
12	Silty clay	5.3	2.50	0.25	50
13	Clay	5.8	3.36	0.33	60
14	Silty clay loam	5.6	4.76	0.48	24
15	Silty clay loam	5.5	5.46	0.60	22

Mineralizable N (NH_4^+) by anaerobic incubation method

Ten g of soil was transferred to a test tube containing 15–20 ml water to give a standing water layer of 2–3 cm. The test tube was covered with aluminium foil and incubated at 30°C for 2 weeks in an anaerobic incubator. This method is essentially the same described by Waring and Bremner[8] with the modification that after incubation samples were extracted with 2 *M* KCl, and a 20-ml aliquot of the filtered extract was distilled with MgO to determine ammonium released[6] as suggested by Sahrawat and Ponnamperuma[7]. All the analyses and experiments reported were done at least in duplicate. Relationships between the ammonium released by acid dichromate oxidation and organic C and total N contents of soils, and the mineralizable N (NH_4^+-N) produced in soils under waterlogged conditions were worked out.

Results and discussion

The amounts of ammonium released in soils by oxidative action of acid dichromate (during simultaneous determination of organic C) ranged from 89 to 785 mg/kg of soil and formed 6.3 to 18.0% of total N contents. On the other hand ammonium produced in soils under waterlogged conditions ranged from 15 to 438 mg/kg of soil and the mineralizable N constituted 2.3 to 8.4% of total N (Table 2).

It was further found that the NH_4^+ released by acid dichromate was highly correlated with the mineralizable N released under waterlogged conditions (r = 0.985**). Also the mineralizable N released by acid dichromate was highly

Table 2. Potentially mineralizable N in soils determined by acid dichromate oxidation and anaerobic incubation methods

Soil No.	Acid dichromate method		Anaerobic incubation	
	mg NH_4^+-N/kg	% of total N	mg NH_4^+-N/kg	% of total N
1	102	17.0	20	3.3
2	108	18.0	15	2.5
3	89	12.7	17	2.4
4	112	14.0	21	2.6
5	115	12.8	24	2.7
6	102	10.2	23	2.3
7	150	13.6	44	4.0
8	145	8.5	63	3.7
9	181	10.1	49	2.7
10	220	12.2	103	5.7
11	132	6.3	52	2.5
12	304	12.2	169	6.8
13	388	11.8	279	8.4
14	552	11.5	315	6.6
15	785	13.1	438	7.3

Table 3. Correlations between NH_4^+ released by oxidative action of acid dichromate and organic C, total N, and mineralizable N released during anaerobic incubation (N = 15).

Parameter compared	Correlation coefficient (r)
Mineralizable N (anaerobic incubation)	0.985**
Total N	0.976**
Organic C	0.969**

** Significant at 1% level

correlated with organic C (r = 0.969**) and total N (r = 0.976**) contents of soils (Table 3). This is mainly due to the fact that both organic C and total N content of soil are highly correlated with mineralizable N released under waterlogged conditions (Table 4) and also with NH_4^+ released by oxidative action of dichromate. Also because organic C and total N contents of soils were very highly correlated (r = 0.996**), which are indexes of organic matter which is the source of mineralizable N measured by the incubation as well as by the acid dichromate methods. These results suggest that the ammonium released by the oxidative active of acid dichromate as well as the ammonium released under anaerobic incubation are derived from the same fraction of organic matter. Since acid dichromate used for organic C determination, also releases NH_4^+ from the soil organic N pool and organic C is an index of potentially mineralizable N released under waterlogged conditions (Table 4), the highly significant relationship between acid dichromate-N and mineralizable N released during waterlogged incubation are obviously due to the fact that both methods derive the mineral N from the same source of organic matter as measured by organic C and total N contents of soils. Regression analyses of the data showed that the NH_4^+ released by acid dichromate (Acid dichromate-N) accounted for 97% of the variation in the NH_4^+ released during anaerobic incubation of the soils with the following equation:

Acid dichromate-N = 69.6 + 1.5 mineralizable N
Variance = 97.0%

Table 4. Correlations between NH_4^+ released under waterlogged incubation with organic C and total N contents of soils (n = 15)

Parameter compared	Correlation coefficient (r)
Organic C	0.973**
Total N	0.973**

** Significant at 1% level

Similarly, Acid dichromate-N accounted for most of the variation in organic C and total N contents of soils by the following regression equations:

Acid dichromate-N = $-32 + 132.0$ organic C
Variance = 94.0%

Acid dichromate-N = $-9.1 + 1219.6$ Total N
Variance = 95.0%

These results indicate the potential of acid dichromate-N for predicting the pools of mineralizable N in soils. Though in the present study the technique has been employed for predicting potentially mineralizable N in wetland rice soils but the method may be found suitable for upland soils as well.

In conclusion the method described is a simple adaptation of the Walkley-Black method used for determination of organic C and the modified method described can be conveniently used for simultaneous estimation of organic C and potentially mineralizable in the same soil sample. The method has the capability of handling a large number of soil samples for routine adaption.

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