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EVALUATION OF SOME CHEMICAL INDEXES FOR PREDICTING MINERALIZABLE NITROGEN IN TROPICAL RICE SOILS

KEY WORDS: Anaerobic incubation, oxidative release, organic matter, acid dichromate, acid permanganate, alkaline permangnate, hydrogen peroxide.

K.L. Sahrawat[1]
The International Rice Research Institute, Los Baños, Philippines

ABSTRACT

Five chemical indexes were evaluated for predicting mineralizable nitrogen in 39 rice soils from the Philippines having a wide range in texture, pH and organic matter. The indexes evaluated were based on oxidative release of soil ammonium-N by alkaline permanganate, acid permanganate, acid dichromate, hydrogen peroxide and dilute sulfuric acid. The amounts of mineralizable N obtained by the chemical indexes were correlated to varying degrees with those determined by anaerobic incubation methods. The basis of good agreement for mineralizable N determined by chemical and biological indexes appears to be due to the fact that both derive the mineral N from the pool of organic nitrogen in soils. The support for this conclusion was obtained by highly significant correlations between mineralizable N determined by chemical and biological indexes with soil organic C and total N contents of soils. These results alongwith other evidence suggest that the chemical indexes may be useful for predicting pools of mineralizable N in wetland rice soils.

INTRODUCTION

Mineralizable nitrogen in wetland rice soils plays an important role in nitrogen nutrition of rice because about 50 to 75% of the total N taken even by a fertilized rice crop comes from the soil mineralizable N pool[5]. Also for efficient and judicious use of fertilizer nitrogen, it is imperative to know the nitrogen supplying capacity of soils. It has been recognized that development of laboratory indexes for predicting soil nitrogen availability is an important component of research for efficient use of fertilizer nitrogen. A large body of literature that has accumulated on the subject is a testimony to the research efforts directed towards development of suitable N availability indexes for crops including rice[3,6,11,13,14].

Many researchers have suggested that the ammonium released by anaerobic incubation of soils for short periods of 6 to 14 days is a good measure of available N to wetland rice[6,12-14, 16,17,21,24]. The incubation tests used though useful in prediction of soil mineralizable N pool are time consuming and the results are affected by the pretreatment of the soil samples prior to carrying out incubation tests[3]. On the other hand chemical indexes are rapid, and easily adaptable to situations where a large number of samples are to be handled. However, Chemical indexes are open to criticism in that they do not simulate the real world situation for biological mineralization of soil organic N. Additionally, they extract a fraction of the total N, which may or may not be liable for biological mineralization. The best approach however, will be to develop chemical indexes that are simple and inexpensive and compare them with the potentially mineralizable N obtained by the incubation tests. The best chemical indexes selected on the basis of their relationship with mineralizable N can be further tested to predict N availability to crops. Relatively less work has been to evaluate and develop simple and rapid chemical indexes for predicting potentially mineralizable N in wet land rice soils[6].

The objectives of this study were to evaluate five promising chemical indexes of mineralizable N with 39 rice soils from the Philippines, having a wide range in soil properties. Relationships between the available N values obtained by the chemical indexes and the mineralizable N released under waterlogged conditions were worked out. Additionally, the correlations between chemical indexes and organic C and total N contents of soils were also worked out. Because previous work has demonstrated that mineralizable N released under waterlogged conditions is highly positively correlated to organic C and total N contents[10,12].

MATERIALS AND METHODS

Soils

Soils used were surface (0-15 cm) samples collected shortly before the study from the rice growing parts of the Philippines. The soil samples were air-dired and ground to pass through a 2-mm sieve before use. Some important properties of the soils are given in Table 1 and show that the soils used had a wide range in pH (4.3 to 7.9), organic C content (0.63 to 5.46%), total N content (0.06 to 0.60%), clay content (12 to 71%), cation exchange capacity (CEC) (7.0 to 51.3 m.e./100g of soil) and C/N ratio (7.9 to 15.9).

For soil analyses reported in Table 1, pH was measured using a soil to water ratio of 1:1 by a blass electrode, organic C was determined by the method of Walkley and Black[22], total N by the semi-micro-Kjeldāhl method of Bremner[2]. CEC and particle size analysis were done as described by Chapman[7] and Day[8] respectively.

Incubation methods

1. Anaerobic incubation at 30°C for 2 weeks: The method used was essentially the one described by Waring and Bremner[23] with some modifications. Ten g soil sample was transferred to a test tube (16 x 2 cm) containing

Table 1

Analysis of soils

Soil No.	Texture	pH (1:1)	Org.C (%)	Total N (%)	C/N	C.E.C (m.e/100g)	Clay (%)
1	Clay	4.5	1.54	0.17	9.0	23.5	62
2	Clay	4.3	1.94	0.18	10.8	16.5	71
3	Clay	5.3	1.48	0.16	9.2	30.3	57
4	Clay	4.4	1.98	0.20	9.9	30.5	53
5	Clay	5.4	2.44	0.31	7.9	36.2	49
6	Clay	5.8	3.36	0.33	10.2	43.0	60
7	Silty clay loam	5.5	5.46	0.60	9.1	44.3	22
8	Silty clay loam	5.6	4.76	0.48	9.9	40.9	24
9	Clay	4.7	2.42	0.26	9.3	36.8	74
10	Silty clay loam	6.4	1.76	0.18	9.8	29.3	33
11	Clay loam	7.4	1.97	0.18	10.9	35.9	33
12	Silty clay loam	5.5	2.05	0.16	12.8	37.7	27
13	Silty clay	6.9	1.69	0.16	10.6	36.2	47
14	Clay	5.1	0.93	0.08	11.6	25.5	56
15	Clay	4.8	0.95	0.08	11.9	23.9	55
16	Clay	7.0	1.89	0.16	11.8	45.4	68
17	Clay	6.4	0.83	0.08	10.4	35.5	60
18	Clay	4.8	1.42	0.15	9.5	18.2	68
19	Clay	5.6	1.15	0.10	11.5	51.3	68
20	Clay	6.6	2.14	0.21	10.2	50.8	68
21	Silty Clay loam	7.2	0.84	0.07	12.0	39.5	41
22	Silty loam	7.5	0.63	0.06	10.5	35.5	23
23	Silt loam	7.9	0.63	0.06	10.5	35.5	23
24	Silty clay	5.7	1.03	0.09	11.4	36.1	48
25	Silty clay loam	7.0	0.91	0.08	11.4	42.3	44
26	Silty clay	5.7	0.83	0.06	13.8	33.8	47
27	Silty clay loam	4.8	1.11	0.07	15.9	27.0	42
28	Silty clay loam	6.1	1.09	0.08	13.6	25.8	30
29	Clay	4.9	1.63	0.13	12.5	44.3	62
30	Silt loam	5.2	0.72	0.06	12.0	9.9	13
31	Silt loam	6.5	1.89	0.16	11.8	20.2	24
32	Clay loam	6.5	0.75	0.08	9.4	30.5	35
33	Sandy loam	4.7	0.65	0.06	10.8	8.6	15
34	Loam	5.0	0.65	0.06	10.8	7.0	12
35	Loam	4.9	0.77	0.07	11.0	8.6	19
36	Silty clay loam	5.3	1.36	0.11	12.4	30.5	32
37	Clay	5.3	1.30	0.11	11.8	33.5	51
38	Silty clay	6.5	1.50	0.13	11.5	34.3	42
39	Silty clay	5.3	2.50	0.25	10.0	40.9	50

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. After incubation period the soil sample was extracted with 2N KCl keeping a soil to solution ratio of 1:10 and a 20-ml aliquot of the filtered extract was distilled with MgO to determine amount of ammonium released[4]. Direct distillation of the incubated soil samples in presence of KCl with MgO as suggested by Waring and Bremner[23] was not followed as it inflated the values of NH_4^+ released probably due to hydrolysis of soil organic N[16].

2. Anaerobic incubation at 40°C for 1 week: The soil samples in this method were incubated at 40°C for 1 week otherwise rest of the procedure followed was identical with the first method.

Chemical methods

1. Release of ammonium from the soil organic N by alkaline permanganate digestion (Alkaline $KMnO_4$-N): The method described by subbiah and Asija[20] was adapted with some modifications.

Five g soil sample was transferred to a 800-ml distillation flask, and 25 ml of 0.32% $KMnO_4$ solution, 25 ml of 2.5% NaOH, 5 ml of water and 2 drops of mineral oil were added. The flask was connected to the distillation unit and the distillation carried out at a slow rate to collect about 20 ml of the distillate in 2% boric acid-indicator mixture in about 15 minutes. NH_4^+-N in the distillate was determined by titration with 0.05 N H_2SO_4. Some soils showed intense bumping without the addition of mineral oil. Addition of a few drops of mineral oil or liquid paraffin is recommended for soils with high content of organic matter.

2. Release of ammonium from the soil organic N by oxidation with the acid permanganate (Acid $KmnO_4$-N): The method followed is essentially the one described by Stanford and Smith[19].

One g of soil sample was shaken with 25 ml of 0.1 N $KMnO_4$ solution in 1N H_2SO_4 for 1 h in an end-over-end shaker in 100 ml polycarbonate tube. After centrifugation, the extract was steam distilled with 50% (w/w) aqueous solution of NaOH. In some soils, filtration was required after centrifugation to obtain clear extracts. The ammonium distilled was absorbed in boric acid and titrated with 0.02 N H_2SO_4 to determine the amount of NH_4^+ released. To obtain the net amount NH_4^+ released by the oxidative action of $KMnO_4$ the amount of NH_4^+ extracted by 1N H_2SO_4 was subtracted. Several modifications of the method were followed to separate out the contribution of permanganate and acid.

3. Release of NH_4^+ to release of NH_4^+-N from the soil organic N pool by 1N H_2SO_4 extraction (H_2SO_4-N): One g of soil sample was shaken for 1 h with 25 ml of 1 N H_2SO_4 and the amount of NH_4^+ in the extract was determined as described under method 2.

4. Oxidative release of NH_4^+ from soil organic N by acid dichromate extraction (Acid $K_2Cr_2O_7$-N): This method is based on my previous work[13,14] and measures release of NH_4^+ by shaking 1 g of soil sample with 25 ml of 0.1 N $K_2Cr_2O_7$ in 1N H_2SO_4 for 1 h in an end-over-end shaker in 100 ml polycarbonate tube. The extract was centrifuged, filtered if necessary and the entire extract was steam distilled with 50% NaOH and the ammonia absorbed in boric acid was titrated with 0.02 N H_2SO_4 to determine NH_4^+ released.

5. Release of NH_4^+ from soil organic N by oxidative action of hydrogen peroxide (H_2O_2-N): Taking leads from my earlier work[13], the following procedure was adapted.

Five g soil sample was placed in a 125-ml conical flask and 5 ml of 30% H_2O_2 (Fisher Chemicals, USA) was added to the soil from a pipette at the same time the flask was swriled gently. After allowing the reactants in the flask for about 1 h., the NH_4^+ released was extracted by shaking the soil samples with 50 ml of 2 M KCl solution for 1 h. NH_4^+ in the filtered extract was determined by distillation of a 25 ml aliquot with MgO as described earlier.

All the incubation tests to determine mineralizable N were carried out in triplicate and the determination of available N by the chemical methods were done in duplicate.

Correlations between different chemical indexes of available N and organic, total N, and mineralizable N released at 30°C (2 weeks) and 40°C (1 week) were worked out.

RESULTS AND DISCUSSION

The amounts of potentially mineralizable N determined by the two incubation methods and 5 chemical indexes provided a wide range in available N (Table 2). The mineralizable N in the soils determined by the anaerobic incubation at 30°C for 2 weeks ranged from 17 to 428 ppm. Similarly, the NH_4^+-N released at 40°C during 1 week incubation ranged from 13 to 457 ppm (Table 2). The chemical indexes too provided a range in available N which varied from as low as 7 ppm (H_2SO_4-N) to as high as 1093 ppm (H_2O_2-N). This is expected in view of the diversified soil samples used in the study which with a wide range in organic matter content. The chemical indexes showed highly significant correlations with mineralizable N released at 30°C (2 weeks) or at 40°C (1 week) (Table 3). The NH_4^+ released in these soils under waterlogged conditions showed highly positive correlations with organic C and total N contents of soils (Table 4).

As observed by stanford and Smith[19], the NH_4^+-N released by acid permanganate from soil residues after pre-extraction with 1N H_2SO_4 was a better and more precise measurement of potentially mineralizable N the results of the present study have also shown that acid $KMnO_4$ extractable-N after pre-extraction with acid showed better correlations with the

TABLE 2

Amounts of available N (µg/g of soil) in 39 soils determined by 2 incubation and 5 chemical methods.

Soil No.	Incubation at 30°C	Incubation at 40°C	Alkaline $KMnO_4$-N	*Acid $KMnO_4$-N	H_2SO_4-N	Acid $K_2Cr_2O_7$-N	H_2O_2-N
1	63	49	249	81	44	48	301
2	75	66	332	95	37	66	213
3	73	70	205	77	40	44	264
4	82	106	226	66	33	33	506
5	332	366	322	110	77	95	636
6	279	315	336	99	44	59	677
7	428	522	397	139	62	110	1093
8	315	457	359	128	51	92	532
9	147	121	246	110	40	59	833
10	49	98	189	92	22	62	193
11	103	158	192	84	37	44	94
12	118	156	188	88	59	66	332
13	50	54	265	92	37	48	141
14	32	19	169	73	37	15	183
15	36	17	169	59	59	29	136
16	47	75	182	70	26	55	112
17	24	34	150	70	33	11	50
18	39	46	227	103	59	37	150
19	23	68	164	66	22	22	29
20	52	163	153	66	22	22	132
21	17	13	127	70	7	12	29
22	17	13	151	70	22	37	33
23	20	14	146	70	15	33	35
24	24	45	134	66	11	29	86
25	21	13	162	59	44	29	26
26	19	19	134	48	29	13	106
27	19	54	137	70	22	18	356
28	24	20	115	62	48	15	53
29	41	42	151	88	66	33	119
30	26	26	113	59	26	22	97
31	54	94	171	110	37	37	167
32	20	23	111	51	44	15	129
33	22	21	145	62	40	37	125
34	41	44	149	70	22	22	110
35	23	31	151	66	40	29	141
36	44	41	172	81	33	48	321
37	41	41	154	70	37	29	196
38	28	51	172	73	37	18	348
39	169	242	227	112	40	66	481

*Acid $KMnO_4$ extractable-NH_4^+ from soil residues left after pre-extraction with H_2SO_4.

TABLE 3

Correlations between chemical indexes of available N and mineralizable N released at 30°C (2 weeks) and 40°C (1 week).

Chemical index compared	Correlation coefficient (r) with Min. N (30°C)	 Min. N (40°C)
Alkaline $KMnO_4$-N	0.859^{**}	0.812^{**}
‡Acid $KMnO_4$-N	0.800^{**}	0.788^{**}
H_2SO_4-N	0.541^{**}	0.457^{**}
Acid $K_2Cr_2O_7$-N	0.858^{**}	0.828^{**}
H_2O_2-N	0.855^{**}	0.795^{**}

** = Significant at the 1% level

‡ = Acid $KMnO_4$ extractable NH_4^+ from soil residues left after pre-extraction with H_2SO_4.

TABLE 4

Correlations between mineralizable N and organic C and total N content of soils (n = 39).

Parameters compared	Correlation coefficient (r)
Min-N (30°C, 2 weeks) vs Org. C	0.909^{**}
Min-N (30°C, 2 weeks) vs Total N	0.940^{**}
Min-N (40°C, 1 week) vs Org. C	0.938^{**}
Min-N (40°C, 1 week) vs Total N	0.952^{**}

** = Significant at the 1% level.

mineralizable N released under anaerobic incubation (Table 5). Additionally, it was indicated that acid $KMnO_4$-N, after pre-extraction with acid also showed the better correlations with organic C and total N contents of soils as compared to the acid $KMnO_4$ extractable-N obtained without pre-extraction with acid or when the extraction was done on two sets of samples separately and the acid extractable N was subtracted from the N extracted by acid

TABLE 5

Relationships between acid permanganate extractable ammonium with and without acid pre-extraction with organic C and total N contents, and mineralizable N in soils (n = 39).

Conditions of acid $KMnO_4$ extractable NH_4^+	Parameter compared	Correlation co-efficient (r)
1. Acid $KMnO_4$-N after pre-extraction with H_2SO_4	Organic C	0.839**
	Total	0.845**
	Min-N (30°C)	0.800**
	Min-N (40°C)	0.788**
2. Acid $KMnO_4$-N without pre-extraction with H_2SO_4	Organic C	0.694**
	Total N	0.719**
	Min - N (30°C)	0.743**
	Min-N (40°C)	0.747**
3. (Acid $KMnO_4$-N) - (Acid - N)	Organic C	0.512**
	Total N	0.531**
	Min-N (30°C)	0.484**
	Min-N (40°C)	0.511**

** = Significant at the 1% level.

$KMnO_4$ to obtain the net amount of NH_4^+ released by the oxidative action of $KMnO_4$ (Table 5).

However, this pre-extraction with acid was found unnecessary in case of acid $K_2Cr_2O_7$ method. As indicated earlier, acid dichromate method developed from our earlier studies, where it was shown that organic C estimated by the Walkley-Black method using acid dichromate oxidation was highly correlated to the mineralizable N released during anaerobic incubation of a large number of rice soils[12,16]. It was visualized that the ammonium released by the oxidative action of acid dichromate might be also related to the mineralizable N in the wetland rice soils. This study supports this hypothesis, because acid $K_2C_2O_7$-N showed highly positive correlations with organic C, total N and mineralizable N. It may be worth investigation to combine the determination of organic C and potentially mineralizable N by acid dichromate oxidation in a single sample to improve the rapidity and make the method more adaptable to routine analysis.

It was further indicated from the relationships between the chemical indexes of available N and organic C and total N contents of soil, that the alkaline $KMnO_4$ method provided the highest correlation coefficients with organic C ($r = 0.855^{**}$) and total N ($r = 0.882^{**}$). Similarly acid $K_2Cr_2O_7$-N, acid $KMnO_4$-N and H_2O_2-N were also highly correlated to the organic C and total N contents of soils, H_2SO_4-N was also correlated to the organic C and total N status of soils but gave lower correlation coefficients than the other chemical indexes (Table 6).

Based on the linear correlations between chemical indexes and mineralizable N (Table 3), it was demonstrated that alkaline $KMnO_4$ acid $K_2Cr_2O_7$, and H_2O_2-N and acid $KMnO_4$-N showed considerable potential in prediction of potentially mineralizable N released under anaerobic incubation of soils at 30°C for 2 weeks or at 40°C for 1 week. As with organic C and total N, H_2SO_4-N showed poor correlations with mineralizable N also (Table 6).

Recently, Stanford[18] and Stanford and Smith[19] have extensively evaluated the alklaine permanganate and acid permanganate extraction methods for prediction of potentially mineralizable N using a large number of soils representing major agricultural regions of the United States and concluded that the alkaline permanganate methods used by various researchers as well as modifications evaluated in their study offered a less precise and reliable basis for prediction of potentially mineralizable N than did the acid permanganate extraction for measuring oxidative release of NH_4-N. However, results of the present study indicate the superiority of alkaline $KMnO_4$-N over acid $KMnO_4$-N. The difference in these results perhaps can be explained in view of the different biological methods used for measuring soil mineralizable N. While Stanford[18] and Stanford and Smith[19] used mineral N released under aerobic conditions as the potentially

TABLE 6

Correlations between chemical indexes of available N and organic C and total N contents of soil (n = 39).

Chemical index compared	Correlation coefficient (r) with	
	Organic C	Total N
Alkaline $KMnO_4$-N	0.855^{**}	0.882^{**}
‡Acid $KMnO_4$-N	0.839^{**}	0.845^{**}
H_2SO_4-N	0.440^{**}	0.461^{**}
Acid $K_2Cr_2O_7$-N	0.830^{**}	0.855^{**}
H_2O_2-N	0.814^{**}	0.840^{**}

** = Significant at the 1% level

‡ = NH_4^+ extracted by acid $KMnO_4$ from soil residues after pre-extraction with H_2SO_4.

mineralizable N, I used NH_4^+ released under anaerobic incubation of soils as the mineralizable N.

Acid extractants have been effectively used for prediction of mineralizable N in acid soils as indicated by the study of Nommik[9], who reported that

phosphoric acid from a large number of acid forest soils in Sweden was highly correlated with the mineralizable N released during aerobic incubation. These results indicate the potentiality of acid $K_2Cr_2O_7$ and acid $KMnO_4$ techniques for prediction of mineralizable N in wetland rice soils.

Alkaline $KMnO_4$ method is extensively used especially in India (see Sahrawat and Burford[15]) and in one of the earlier studies by Subbiah and Asija[20], it was shown that the alkaline $KMnO_4$-N was highly positively correlated with the mineralizable N released during 45 days. These results provide further evidence to show that the alkaline $KMnO_4$-N is a good index for prediction of mineralizable

Most of the studies reported on chemical indexes of mineralizable N pertain to the upland soils involving aerobic incubation techniques. It is also evident that the chemical indexes used measure release of NH_4^+ from soil organic pool by oxidation and or hydrolysis. Wetland rice utilizes a large part of the N in NH_4^+ form and this is usually predicted by mineralizable N released during anaerobic incubation of soils. It appears logical that a chemical index that extracts NH_4^+ from a specific fraction of soil organic matter which is the source of NH_4^+-N released during anaerobic incubation should provide a good index of mineralizable N. Among the chemical indexes evaluated in this study those based on oxidative release of NH_4^+ by alkaline permanganate, acid dichromate, hydrogen peroxide, and acid permanganate seem promising. Our results with a large number of wetland rice soils from the Philippines have demonstrated that organic C and total N are good indexes of potential mineralizable N released under anaerobic incubation at 30°C for 2 weeks[10,12]. The results of the present study have clearly shown that chemical methods that are correlated well with organic C and total N contents of soils are also good indexes of mineralizable N released during anaerobic incubation. This is mainly due to the fact that both the chemical and biological indexes derive mineralizable N from the same pool of organic N. Stanford and Smith[19] have aptly suggested that the chemical indexes that extract NH_4^+ from soil organic N that is most readily susceptible to biological mineralization should provide a good estimate of mineralizable N pool in soils. The results of this study offer encouragement for the use of chemical indexes for prediction of mineralizable N in wetland rice soils.

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One g of soil sample was shaken with 25 ml of 0.1 N $KMnO_4$ solution in 1N H_2SO_4 for 1 h in an end-over-end shaker in 100 ml polycarbonate tube. After centrifugation, the extract was steam distilled with 50% (w/w) aqueous solution of NaOH. In some soils, filtration was required after centrifugation to obtain clear extracts. The ammonium distilled was absorbed in boric acid and titrated with 0.02 N H_2SO_4 to determine the amount of NH_4^+ released. To obtain the net amount NH_4^+ released by the oxidative action of $KMnO_4$ the amount of NH_4^+ extracted by 1N H_2SO_4 was subtracted. Several modifications of the method were followed to separate out the contribution of permanganate and acid.

3. Release of NH_4^+ to release of NH_4^+-N from the soil organic N pool by 1N H_2SO_4 extraction (H_2SO_4-N): One g of soil sample was shaken for 1 h with 25 ml of 1 N H_2SO_4 and the amount of NH_4^+ in the extract was determined as described under method 2.

4. Oxidative release of NH_4^+ from soil organic N by acid dichromate extraction (Acid $K_2Cr_2O_7$-N): This method is based on my previous work[13,14] and measures release

25 ml of 0.1 N $K_2Cr_2O_7$ in 1N H_2SO_4 for 1 h in an end-over-end shaker in 100 ml polycarbonate tube. The extract was centrifuged, filtered if necessary and the entire extract was steam distilled with 50% NaOH and the ammonia absorbed in boric acid was titrated with 0.02 N H_2SO_4 to determine NH_4^+ released.

5. Release of NH_4^+ from soil organic N by oxidative action of hydrogen peroxide (H_2O_2-N): Taking leads from my earlier work[13], the following procedure was adapted.

Five g soil sample was placed in a 125-ml conical flask and 5 ml of 30% H_2O_2 (Fisher Chemicals, USA) was added to the soil from a pipette at the same time the flask was swriled gently. After allowing the reactants in the flask for about 1 h., the NH_4^+ released was extracted by

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