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Assay of nitrogen supplying capacity of tropical rice soils

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Key words Acid dichromate Acid permanganate Alkaline permanganate Anaerobic incubation H_2O_2 oxidation N uptake of rice Organic C Oxidative release Soil N availability index Total N.

Summary The nitrogen supplying capacity of 39 wetland rice soils evaluated by two anaerobic incubation methods and six chemical methods was compared with N uptake of IR 26 rice grown on these soils under flooded conditions in a greenhouse pot study. The uptake of N by rice correlated highly with the N supplying capacity determined by anaerobic incubation methods involving incubation of soils at 30°C for 2 weeks ($r = 0.84^{**}$) or at 40°C for 1 week ($r = 0.82^{**}$) as well as with the organic carbon ($r = 0.82^{**}$) and total N ($r = 0.84^{**}$) contents of soils. Among the chemical indexes, available N determined by the oxidative release of soil N by alkaline permanganate, acid permanganate, acid dichromate and hydrogen peroxide also provided good index of soil N availability to rice. According to these results soil organic carbon and total N contents seem to be good indexes of available nitrogen in tropical wetland rice soils.

Introduction

The current fertilizer shortage coupled with high prices due to the energy crisis have stimulated research on the efficient use of fertilizer nitrogen. For efficient and judicious use of fertilizer nitrogen, it is imperative to assess the nitrogen supplying power of soils. Thus development of laboratory indexes for predicting soil nitrogen availability to rice is an important component of the research for efficient and economic use of fertilizer nitrogen.

Numerous biological and chemical methods for measuring soil nitrogen availability to plants have been reviewed by Bremner[5], Robinson[20] and Chang[6]. According to several workers, the incubation methods, though time consuming provide a good index of soil nitrogen availability to plants[5,6,9,10,12,14,21,35]. Many researchers have reported that the amount of ammonium released by anaerobic incubation of soils is a good measure of available nitrogen to wetland rice[6,10,15,17,24,25,30,31]. Among the chemical indexes proposed for soil nitrogen availability to rice include measurement of organic carbon and total N contents[1,21,25] or of mineral nitrogen released by extraction or digestion with neutral, acid or alkaline reagents[5,11,20,27,28,29,30]. The ammonium released from soil organic matter by boiling with alkaline permanganate was proposed by Truog[32] as an index for soil nitrogen availability. This method has been modified by several workers[13,22,28,30] and has been extensively used especially in India for predicting soil N availability to many crops including rice[1,11,16,18,19,26].

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In an earlier communication I reported that organic C and total N contents were good indices of soil N availability to wetland rice grown on eight rice soils from the Philippines[21]. The objectives of the work reported in this paper were to evaluate two incubation methods and six chemical methods for predicting soil N availability to rice grown under flooded conditions on 39 soils having a wide range in pH, organic C, total N and other soil properties.

The two anaerobic incubation methods were those employing incubation at 30°C for 2 weeks or at 40°C for 1 week. The chemical methods tested were: total nitrogen, organic carbon, oxidative release of NH_4^+–N from soil organic matter by alkaline permanganate[30], acid permanganate[29], acid dichromate and hydrogen peroxide.

Materials and methods

Soils

The 39 soils used were surface (0–15 cm) samples collected shortly before the study from different rice growing parts of the Phillipines. Soils with growth-limiting factors like iron toxicity, excess reduction products and severe mineral deficiencies were excluded. Table 1 shows that the soils used in the study differed markedly in pH (4.3 to 7.9), organic carbon content (0.63 to 5.46%), total nitrogen content (0.06 to 0.60%), cation exchange capacity (CEC) (70 to 513 m.mol/kg of soil), clay content (12 to 71%) and C/N ratio (7.9 to 15.9).

About 30 kg of soil in bulk was collected from each site under field moist conditions. The samples were air dried and screened through a 5 mm sieve and mixed thoroughly. A portion of each sample was ground to pass a 2 mm sieve for use in the laboratory tests. For soil analyses reported in Table 1, pH was measured (1:1 soil to water) by a glass electrode, organic carbon was determined by the method of Walkley and Black[33], total nitrogen by the semi-micro-Kjeldahl method of Bremner[2]. CEC and particle size analysis were done as described by Chapman[7] and Day[8] respectively.

Greenhouse procedure

Four kg of soil (< 5 mm) was placed in 6–1 porcelain pots. The soil was saturated with water and puddled after applying K and P as muriate of potash and triple – superphosphate respectively; each at a rate of 50 mg/kg of soil, and Zn as zinc oxide at 10 mg/kg of soil. Four pregerminated seeds of IR 26 were dibbled in each pot and were later thinned to two plants/pot. The level of soil submergence was increased as the seedlings grew and was maintained at about 5 cm throughout the growing season. There were three replications for each soil.

The plants were grown up to 55 days after seeding and were then cut about 2 cm above the soil surface, washed and dried at 60°C. Dry matter weights were recorded and the plant materials were then ground. Total nitrogen in the ground samples was determined separately for each replication by the micro Kjedahl method[3].

Incubation methods

1. Anaerobic incubation at 30°C for 2 weeks: Ten g of soil was placed in a test tube (16 × 2 cm) containing about 15–20 ml water to give a standing water laver of 2–3 cm. The soil was transferred to the test tube containing water to minimize trapping of air. The test tube was covered with aluminium foil and incubated at 30°C for 2 weeks in an anearobic incubator. This method is essentially the same as described by Waring and Bremner[34] with the modification that after incubation, the soil samples were extracted with 2 *M* KCl keeping a soil to KCl ratio of 1:10 and a 20-ml aliquot of the filtered extract was distilled with MgO to determine the amounts of ammonium released[4]. Direct distillation

Table 1. Analyses of soils used

No.	Soil Texture	pH (1 : 1)	Org. C (%)	Total N (%)	C/N	CEC (m. mol/kg)	Clay (%)
1	Clay	4.5	1.54	0.17	9.0	235	62
2	Clay	4.3	1.94	0.18	10.8	165	71
3	Clay	5.3	1.48	0.16	9.2	303	57
4	Clay	4.4	1.98	0.20	9.9	305	53
5	Clay	5.4	2.44	0.31	7.9	362	49
6	Clay	5.8	3.36	0.33	10.2	430	60
7	Silty clay loam	5.5	5.46	0.60	9.1	443	22
8	Silty clay loam	5.6	4.76	0.48	9.9	409	24
9	Clay	4.7	2.42	0.26	9.3	368	74
10	Silty clay loam	6.4	1.76	0.18	9.8	293	33
11	Clay loam	7.4	1.97	0.18	10.9	359	33
12	Silty clay loam	5.5	2.05	0.16	12.8	377	27
13	Silty clay	6.9	1.69	0.16	10.6	362	47
14	Clay	5.1	0.93	0.08	11.6	255	56
15	Clay	4.8	0.95	0.08	11.9	239	55
16	Clay	7.0	1.89	0.16	11.8	454	68
17	Clay	6.4	0.83	0.08	10.4	355	60
18	Clay	4.8	1.42	0.15	9.5	182	68
19	Clay	5.6	1.15	0.10	11.5	513	68
20	Clay	6.6	2.14	0.21	10.2	508	68
21	Silty Clay loam	7.2	0.84	0.07	12.0	395	41
22	Silty loam	7.5	0.63	0.06	10.5	355	23
23	Silt loam	7.9	0.63	0.06	10.5	355	23
24	Silty clay	5.7	1.03	0.09	11.4	361	48
25	Silty clay loam	7.0	0.91	0.08	11.4	423	44
26	Silty clay	5.7	0.83	0.06	13.8	338	47
27	Silty clay loam	4.8	1.11	0.07	15.9	270	42
28	Silty clay loam	6.1	1.09	0.08	13.6	258	30
29	Clay	4.9	1.63	0.13	12.5	443	62
30	Silt loam	5.2	0.72	0.06	12.0	99	13
31	Silt loam	6.5	1.89	0.16	11.8	202	24
32	Clay loam	6.5	0.75	0.08	9.4	305	35
33	Sandy loam	4.7	0.65	0.06	10.8	86	15
34	Loam	5.0	0.65	0.06	10.8	70	12
35	Loam	4.9	0.77	0.07	11.0	86	19
36	Silty clay loam	5.3	1.36	0.11	12.4	305	32
37	Clay	5.3	1.30	0.11	11.8	335	51
38	Silty clay	6.5	1.50	0.13	11.5	343	42
39	Silty clay	5.3	2.50	0.25	10.0	409	50

of the incubated soil samples in presence of KCl with MgO as suggested by Waring and Bremner[34] was not followed as it resulted in inflated values for ammonium nitrogen[23].

2. Anaerobic incubation at 40°C for 1 week: The procedure was identical with the first method except that the soil samples were incubated at 40°C for one week.

Chemical methods

1. Organic carbon: Organic carbon of the soils was determined by the Walkley and Black method[33].

2. Total nitrogen: Total nitrogen content of the soil samples was determined by the micro Kjeldahl method[2].

3. Release of ammonium nitrogen from the soil organic matter by alkaline permanganate digestion[30]: Five g soil sample was placed in a 800 ml distillation flask and 25 ml of 0.32% $KMnO_4$, 25 ml of 2.5% NaOH, 5 ml of water and 2 drops of mineral oil were added. The distillation was carried at a slow rate for about 15 minutes to collect about 20 ml of the distillate in 2% boric acid-indicator solution. NH_4^+–N in the distillate was determined by titration with 0.025 *M* H_2SO_4. It was observed that distillation of soil with $KMnO_4$ and alkali without liquid paraffin was difficult because of intense bumping.

4. Oxidative release of mineralizable soil N by acid permanganate digestion: This is the method recently proposed by Stanford and Smith[29]. The method involved shaking of 1 g of a soil sample with 25 ml of 0.02 *M* $KMnO_4$ solution in 0.5 *M* H_2SO_4 for 1 hour. After centrifuging or filtration the extract was steam distilled with 50% (W/W) aqueous solution of NaOH. The ammonia distilled was absorbed in boric acid and titrated with 0.01 *M* H_2SO_4. As suggested by the authors, two methods of extracting soil NH_4^+–N were employed. In one, the soil samples were pre-extracted with 0.5 *M* H_2SO_4, the extract discarded and then the samples were extracted with 0.02 *M* $KMnO_4$ solution in 0.5 *M* H_2SO_4 to make allowance for the acid – extractable NH_4^+. In the second method, two separate extractions on two sets of samples were carried out using 0.02 *M* $KMnO_4$ + 0.5 *M* H_2SO_4 and 0.5 *M* H_2SO_4 alone and the amount of NH_4^+ extracted by H_2SO_4 was subtracted from the amount extracted by $KMnO_4$ + H_2SO_4 to obtain the amount of nitrogen released by $KMnO_4$ oxidation.

5. Oxidative release of soil mineralizable N by acid dichromate extraction: Earlier studies in our laboratory showed that the organic carbon content of eight soils correlated well with nitrogen uptake of rice and also with nitrogen mineralized under waterlogged conditions[21]. Since dichromate used for oxidation of soil organic carbon also results in release of NH_4^+ from the organic nitrogen it was thought worthwhile to use this as an index for soil N availability to rice. After some preliminary tests the following method was adopted:

One g of soil was shaken with 25 ml of 0.02 *M* $K_2Cr_2O_7$ solution in 0.5 *M* 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.

6. Release of mineral N following oxidation of soil organic matter by hydrogen peroxide: After preliminary testing in our laboratory, the following method was followed: Five g soil was placed in a 125 ml conical flask and 5 ml of 30% H_2O_2 (Fischer Chemicals) was added slowly to the soil from a pipette at the same time swirling the flask gently. The reactants in the flask were left for about 1 h and then extracted by shaking with 50 ml of 2 *M* KCl solution for 1 h NH_4^+ in the filtrered extracts were determined by distilling a suitable aliquot with MgO.

For a few soils, the NH_4^+ released by KCl after oxidation by hydrogen peroxide was determined by distilling the extract with 50% NaOH. The incubation tests were carried out in triplicate and all other determinations were made at least in duplicate.

For all the methods tested the correlations of available nitrogen values with dry matter weight and nitrogen uptake of rice were worked out.

Results and discussion

In general it was observed that all the methods used for nitrogen availability indexes gave higher correlations with nitrogen uptake than with the dry matter weight of rice (Table 2).

Table 2. Correlation between values of available soil nitrogen by different methods with dry matter yield and N uptale of rice (n = 39)

Method	Correlation coefficient (r)	
	Dry matter wt	N uptake
Organic C, %	0.45**	0.82**
Total N, %	0.46**	0.84**
Incubation, 30°C (2 weeks)	0.40*	0.84**
Incubation, 40°C (1 week)	0.46**	0.82**
Alkaline $KMnO_4$	0.40*	0.81**
Acid $KMnO_4$	0.39*	0.75**
Acid $K_2Cr_2O_7$	0.39*	0.74**
H_2O_2 oxidation	0.46**	0.82**
0.5 *M* H_2SO_4	0.10^{ns}	0.42**

^{ns} = not significant
* = significant at the 5% level
** = significant at the 1% level

Incubation methods

The soils varied widely in the amounts of NH_4^+ released under flooded conditions at 30°C for 2 weeks (17 to 428 ppm) or at 40°C for 1 week (13 to 522 ppm) and the mineralizable NH_4^+ formed 1.4 to 11.8% of the total N in soil. The nitrogen supplying capacity of the soils determined by the incubation methods was highly correlated with the N uptake of rice (Fig. 1).

Chemical methods

Organic carbon content and total nitrogen content of soils were highly correlated with dry matter yields (Table 2) and nitrogen uptake (Fig. 2). These observations are consistent with the findings of others researchers[1,20,21,26] and suggest that simple tests like organic carbon and total nitrogen could be useful as routine methods for predicting soil nitrogen availability for wetland rice.

The correlations of available nitrogen measured by various chemical methods and N uptake of rice are shown in Fig. 2, 3, 4, and 5. The figures show that the values of available nitrogen based on the oxidative release of nitrogen from the soil organic matter by alkaline $KMnO_4$ acid $KMnO_4$ acid $K_2Cr_2O_7$ and H_2O_2 were highly correlated with the nitrogen uptake by rice.

The studies with acid $KMnO_4$ showed that when pre-acid extraction was omitted, the correlation of mineralizable N with nitrogen uptake was poorer ($r = 0.57$**) than when pre extraction with 0.5 *M* H_2SO_4 was used ($r = 0.75$**). The correlation of nitrogen uptake with the values of available nitrogen obtained by extraction with 0.02 *M* $KMnO_4$ in 0.5 *M* H_2SO_4 minus the value of available nitrogen extracted by 0.5 *M* H_2SO_4 was also poor ($r = 0.35$*). The NH_4^+

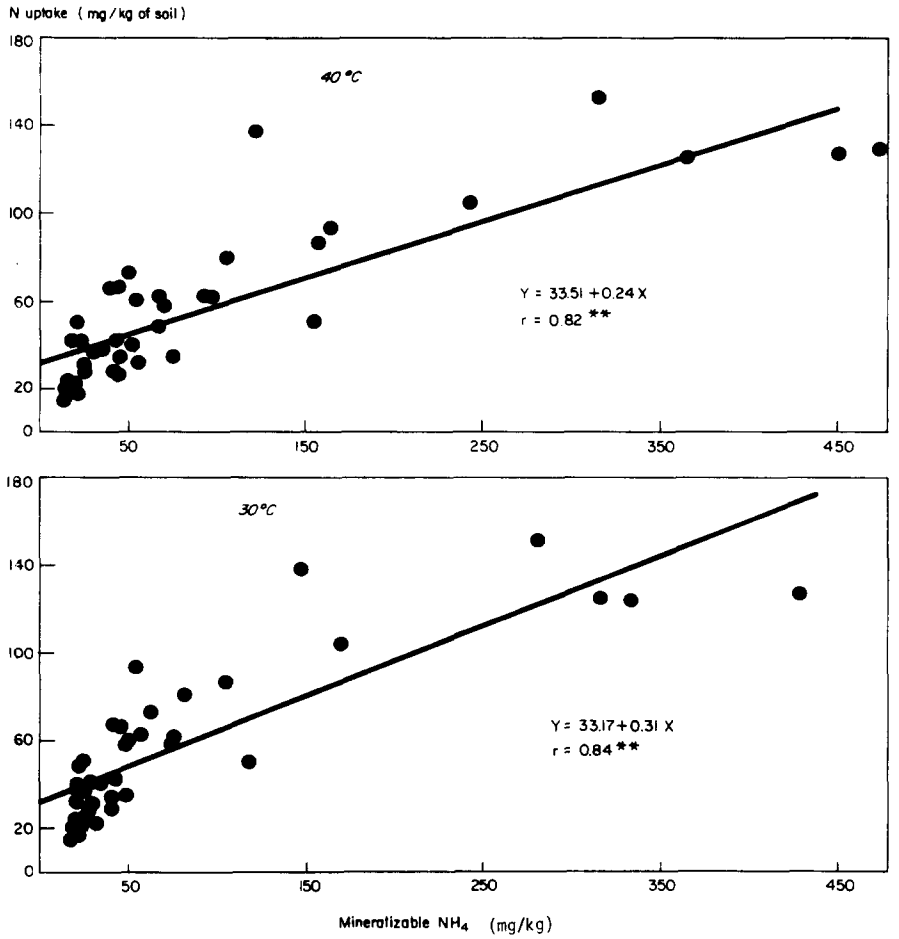

Fig. 1. Relationship between N uptake by rice and NH_4^+ produced by anaerobic incubation at 30°C for 2 weeks and at 40°C for 1 week.

extracted by 0.5 *M* H_2SO_4 was also correlated with nitrogen uptake but the correlation was poor ($r = 0.42^{**}$).

The findings of Stanford and Smith[29] also indicated that the pre-extraction of soil samples with 0.5 *M* H_2SO_4 was essential before extracting the soil samples with acid $KMnO_4$ reagent. The results further confirm the usefulness of this method proposed by Stanford and Smith[29] by providing highly significant correlation between available NH_4^+ released by the oxidative action of acid $KMnO_4$ and uptake of nitrogen by rice crop.

In case of 0.02 *M* $K_2Cr_2O_7$ in 0.5 *M* H_2SO_4 reagent used for extractable nitrogen it was not necessary to have the pre-extraction of soil samples with

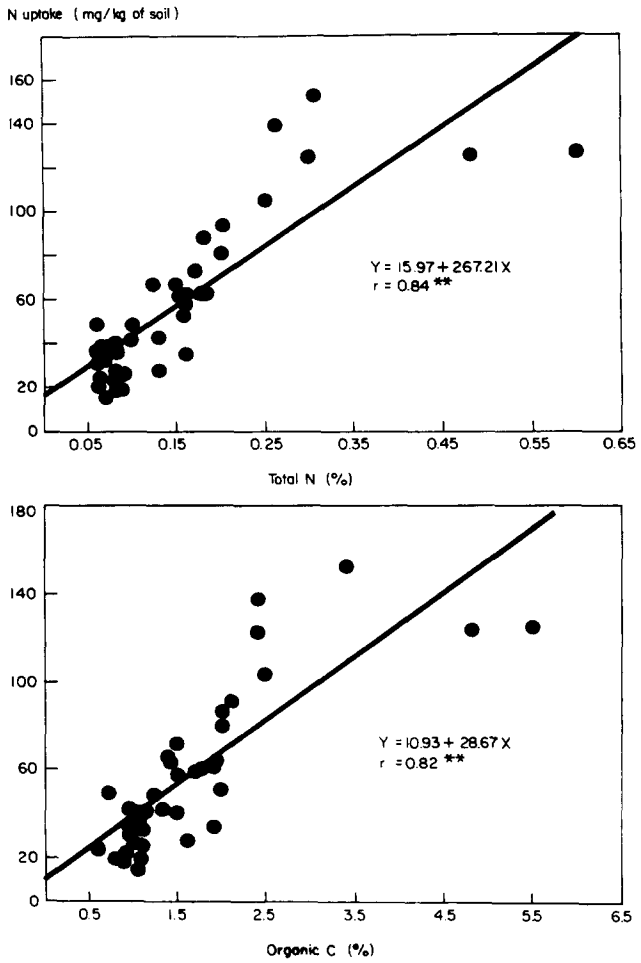

Fig. 2. Relationship between organic C and total N contents of soils and N uptake of rice.

0.5 *M* H_2SO_4 because our preliminary studies indicated that the amounts of NH_4^+extracted by acid $K_2Cr_2O_7$ reagent were similar whether pre-extraction with 0.5 *M* H_2SO_4 was done or not. This makes the method rapid and simple because it involves only a single step extraction. Further studies have indicated that this method can be combined with the method of determining organic carbon by the method of walkley and Black[33] with minor modifications. The titration of excess $Cr_2O_7^{2-}$ left after oxidation of organic matter is done with standard ferrous sulfate solution rather than by ferrous ammonium sulfate. A suitable aliquot from the total volume (after titration with ferrous sulfate) can be distilled with 50% NaOH solution to determine the ammonium released by acid

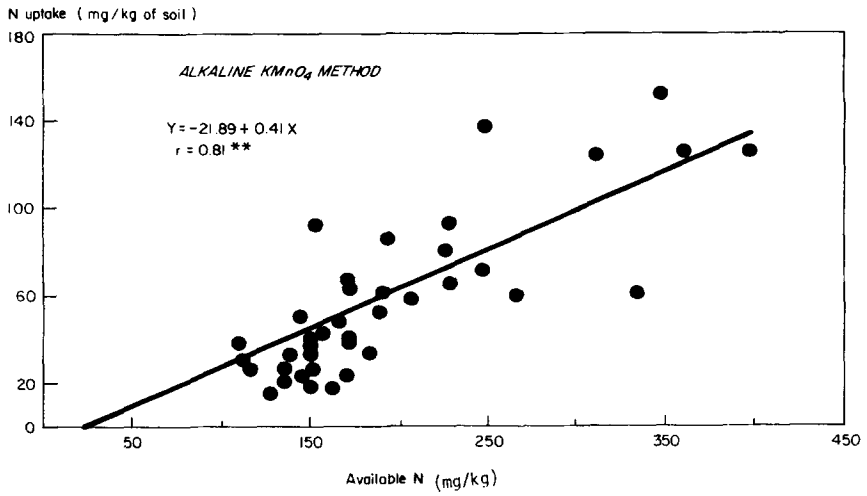

Fig. 3. Relationship between available N values by alkaline $KMnO_4$ method and N uptake of rice.

dichromate. However, further work is needed to find out whether the predictability of soil nitrogen availability could be improved by combining organic carbon plus nitrogen released by oxidation of organic matter by $K_2Cr_2O_7 - H_2SO_4$ over the indexes based on organic carbon or acid $K_2Cr_2O_7$ alone. Our preliminary results offer encouragement for further studies.

Another chemical method which developed from our earlier observations with 8 soils, is based on oxidation of organic matter by hydrogen peroxide[21]. This

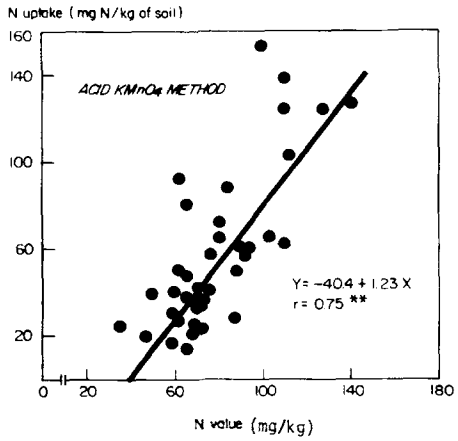

Fig. 4. Relationship between available N values by acid $KMnO_4$ method and N uptake of rice.

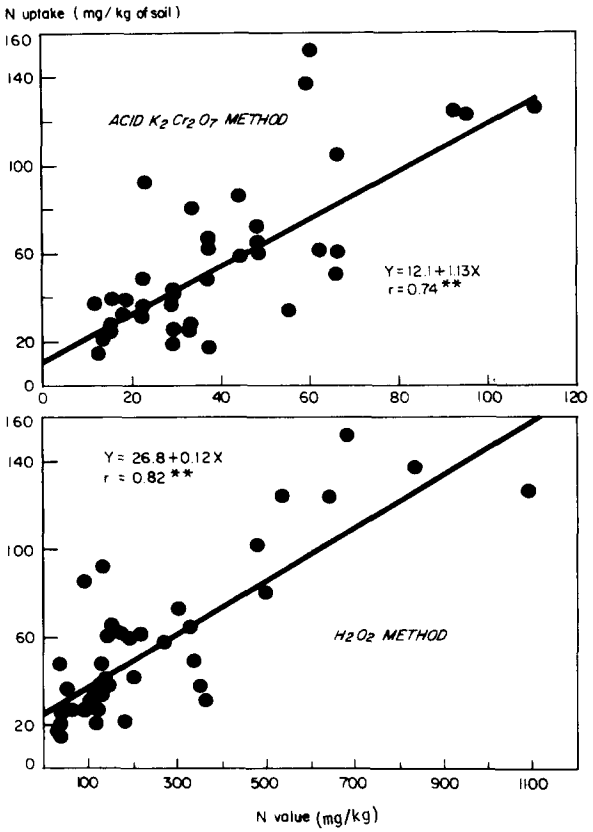

Fig. 5. Relationship between N uptake of rice and available N values by acid $K_2Cr_2O_7$ and H_2O_2 methods.

method too is simple and direct and gave a high correlation with the nitrogen uptake of rice ($r = 0.82$**). The values of H_2O_2-extractable NH_4^+ were very high when 50% NaOH was used as alkalizer to distil NH_4^+ (2–3 times higher) as compared to MgO. Thus MgO distillation method was preferred and was used for correlation studies.

Similarly, the alkaline $KMnO_4$ method was also useful in predicting the availiability of soil nitrogen to rice, giving a correlation coefficient of 0.81** between the available nitrogen by this method and uptake of nitrogen by rice in the greenhouse. These observations are in accord with those made by earlier workers[1,16,18,19,26].

The present study brings out the usefulness of organic carbon and total nitrogen contents for predicting nitrogen availability to lowland rice. Incubation

tests involving anaerobic incubation at 30°C for 2 weeks or at 40°C for 1 week also provided good indexes of nitrogen availability. The oxidative release of NH_4^+ from the soil organic matter with alkaline $KMnO_4$ or acid $KMnO_4$ was found to be highly correlated with nitrogen uptake of rice. In addition this study also proposes two new simple tests based on the oxidative release of soil mineralizable nitrogen by acid dichromate and hydrogen peroxide.

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