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# COMPARISON OF CHEMICAL METHODS OF ASSESSING POTENTIALLY AVAILABLE ORGANIC NITROGEN IN SOIL[1]

KEY WORDS: Phosphate-borate buffer method, KCl method, acid $KMnO_4$ method, alkaline $KMnO_4$ method, $CaCl_2$-autoclave method, $NaHCO_3$ UV method, aerobic incubation method, anaerobic incubation method, N mineralization potential

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## ABSTRACT

We recently developed two rapid and precise chemical methods of assessing potentially available organic N in soils. One method involves determination of the ammonia-N produced by steam distillation of the soil sample with pH 11.2 phosphate-borate buffer solution for 8 min. The other involves determination of the ammonium-N produced by treatment of the soil sample with 2M KCl solution at 100°C for 4 hours. Studies using 33 Brazilian soils showed that the results obtained by these methods were highly correlated with those obtained by anaerobic and aerobic incubation methods of assessing potentially available organic N in soil.

The two methods were further evaluated by applying them to 30 Iowa soils and by comparing their results and those obtained by other chemical methods with the results of the incubation methods considered to be the best laboratory methods currently available for assessment of potentially available organic N in soil. The



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chemical methods used included the acid $KMnO_4$ method, the alkaline $KMnO_4$ method, the $CaCl_2$-autoclave method, and the $NaHCO_3$ UV method. The incubation methods used involved determination of the ammonium-N produced by incubation of the soil sample under anaerobic conditions for 1 week or determination of the (ammonium + nitrate + nitrite)-N produced by incubation of the sample under aerobic conditions for 2 and 12 weeks. The data obtained showed that the results of the two chemical methods evaluated were highly correlated with those obtained by the incubation techniques used for comparison and that the correlations observed with these two methods were higher than those observed with the previously proposed chemical methods. It is concluded that these two rapid and simple methods are the best chemical methods thus far developed for laboratory assessment of potentially available organic N in soil.

## INTRODUCTION

The need for satisfactory laboratory methods of obtaining an estimate of the amount of nitrogen (N) likely to be made available for crop growth by mineralization of soil organic matter during the growing season has long been evident, and numerous biological and chemical methods have been proposed[2-5]. It is generally accepted that the most reliable methods currently available are those involving determination of the inorganic N produced by incubation of the soil sample under aerobic or anaerobic conditions for various times, but these biological methods are not simple and rapid enough for use in soil testing laboratories, and there is an urgent need for a rapid chemical method of assessing potentially available organic soil N that is suitable for use in such laboratories.

Recent work in our laboratory[6-8] led to development of two rapid and precise chemical methods of assessing potentially available organic soil N that are simple enough for use in soil testing laboratories. One method (hereinafter described as the

phosphate-borate buffer method) involves determination of the ammonia-N produced by steam distillation of the soil sample with pH 11.2 phosphate-borate buffer for 8 min. The other (hereinafter described as the KCl method) involves determination of the ammonium-N produced by heating the soil sample with 2M KCl in a stoppered tube at 100°C for 4 hours. These methods were developed by studies using 33 Brazilian soils, which showed that the results obtained by both methods were highly correlated with those obtained by anaerobic and aerobic incubation methods of assessing potentially available organic N in soil, including the anaerobic incubation method recommended in the recent revision of the American Society of Agronomy (ASA) monograph on "Methods of Soil Analysis"[4].

The purpose of the work reported here was to further evaluate these two methods by applying them to 30 diverse Iowa soils and by comparing their results and those obtained by other chemical methods with the results obtained by the incubation methods considered to be the best laboratory methods currently available for assessment of potentially available organic N in soil. The chemical methods used included the acid $KMnO_4$ method proposed by Stanford and Smith[9], an alkaline $KMnO_4$ method proposed by Stanford[10], the $CaCl_2$-autoclave method recommended in the recent revision of the ASA monograph on "Methods of Soil Analysis"[4], the $NaHCO_3$ UV method proposed by Fox and Piekielek[11], and modifications of the KCl method under evaluation. The chemical methods used were also evaluated by comparing their results with the N mineralization potential ($N_o$) values of the 30 Iowa soils studied as calculated by a modification of the procedure described by Stanford and Smith[12].

## MATERIALS

The soils used (Table 1) were surface (0-15 cm) samples of 30 Iowa soils selected to include samples of most of the major soil types in Iowa and obtain a wide range in organic-matter content (0.32-9.10% organic C), total N content (0.030-0.735% N), texture

TABLE 1

Properties of Soils Used

| Soil | | | | Organic | Total | | | |
|---|---|---|---|---|---|---|---|---|
| Series | Subgroup* | pH | CCE+ | C | N | Sand | Silt | Clay |
| | | | | % | | | | |
| Thurman | UHL | 5.4 | 0 | 0.32 | 0.030 | 94 | 3 | 3 |
| Storden | TUT | 8.1 | 20.6 | 0.34 | 0.034 | 51 | 31 | 18 |
| Dickinson | THL | 6.3 | 0 | 0.53 | 0.050 | 90 | 5 | 5 |
| Buckney | EHL | 8.3 | 29.8 | 0.70 | 0.050 | 80 | 6 | 14 |
| Sparta | EHL | 5.5 | 0 | 0.74 | 0.061 | 75 | 16 | 9 |
| Ida | TUT | 8.1 | 10.3 | 0.94 | 0.097 | 9 | 68 | 23 |
| Dickinson | THL | 5.3 | 0 | 1.26 | 0.120 | 58 | 28 | 14 |
| Clarinda | TAL | 5.9 | 0 | 1.41 | 0.139 | 9 | 45 | 46 |
| Clarion | THL | 6.5 | 0 | 1.43 | 0.160 | 62 | 11 | 27 |
| Monona | THL | 6.4 | 0 | 1.62 | 0.156 | 4 | 70 | 26 |
| Shelby | TAL | 5.9 | 0 | 1.66 | 0.211 | 23 | 44 | 33 |
| Fayette | THF | 6.0 | 0 | 1.71 | 0.161 | 4 | 86 | 10 |
| Hayden | THF | 6.3 | 0 | 1.68 | 0.200 | 34 | 50 | 16 |
| Nicollet | AHL | 6.4 | 0 | 1.98 | 0.152 | 46 | 32 | 22 |
| Muscatine | AAL | 6.6 | 0 | 2.05 | 0.220 | 10 | 57 | 33 |
| Marshall | THL | 5.9 | 0 | 2.21 | 0.219 | 2 | 43 | 55 |
| Grundy | AAL | 6.5 | 0 | 2.41 | 0.191 | 5 | 68 | 27 |
| Readlyn | AHL | 6.2 | 0 | 2.44 | 0.190 | 29 | 52 | 19 |
| Wacousta | TPL | 6.8 | 0 | 2.52 | 0.206 | 12 | 59 | 29 |
| Webster | TPL | 6.8 | 0 | 2.63 | 0.210 | 41 | 29 | 30 |
| Galva | THL | 6.7 | 0.4 | 2.97 | 0.251 | 4 | 61 | 35 |
| Webster | TPL | 6.8 | 0 | 3.09 | 0.240 | 35 | 31 | 34 |
| Okoboji | CHL | 7.5 | 0.4 | 3.20 | 0.231 | 22 | 41 | 37 |
| Webster | TPL | 5.8 | 0 | 3.44 | 0.300 | 27 | 42 | 31 |
| Wacousta | TPL | 5.9 | 0 | 3.75 | 0.317 | 16 | 47 | 37 |
| Weller | AHF | 6.0 | 0 | 3.84 | 0.325 | 2 | 77 | 21 |
| Canisteo | TPL | 7.9 | 6.1 | 3.87 | 0.333 | 30 | 39 | 31 |
| Canisteo | TPL | 8.0 | 7.1 | 4.11 | 0.350 | 26 | 37 | 37 |
| Harps | TCL | 7.9 | 12.9 | 5.70 | 0.520 | 27 | 41 | 32 |
| Tillamook | AHT | 4.8 | 0 | 9.10 | 0.735 | 13 | 59 | 28 |

*UHL, Udorthentic Haplustoll; TUT, Typic Udorthent; THL, Typic Hapludoll; EHL, Entic Hapludoll; TAL, Typic Argiudoll; THF, Typic Hapludalf; AHL, Aquic Hapludoll; AAL, Aquic Argiudoll; TPL, Typic Calciaquoll; AHT, Andic Haplumbrept.

+$CaCO_3$ equivalent.

(2-94% sand, 3-86% silt, 3-55% clay), and pH (4.8-8.3). Before use, each sample was air-dried and crushed to pass through a 2-mm screen. The analyses reported in Table 1 were performed as described by Zantua and Bremner[13].

## METHODS OF ASSESSING POTENTIALLY AVAILABLE ORGANIC SOIL NITROGEN

### Chemical Methods

Method 1. This involved determination of organic carbon (C) in the soil sample by the wet oxidation method described by Mebius[14].

Method 2. This involved determination of total nitrogen (N) in the soil sample by the semimicro-Kjeldahl procedure described by Bremner and Breitenbeck[15].

Method 3. This was the KCl method under evaluation. It involves determination of the ammonium-N produced from organic soil N when soil is heated with 2M KCl at 100°C for 4 hours. In this method, 3.0 g of soil are heated with 20 ml of 2M KCl for 4 hours in a stoppered tube placed in a 40-tube block digester maintained at 100°C, and the ammonium-N present after this treatment is determined by estimating the ammonia-N liberated by steam-distilling the soil-KCl mixture with 0.2 g of MgO for 5 min. The ammonium-N present in the soil sample before heating with 2M KCl is determined by estimating the ammonia-N liberated by steam-distilling 3.0 g of soil with 0.2 g of MgO and 20 ml of 2M KCl for 5 min, and the ammonium-N produced from organic soil N by heating the soil sample with 2M KCl is calculated as the difference between the results of these two analyses.

Method 4. This was a modification of method 3 in which the soil sample was heated with 2M KCl in a stoppered tube at 95°C for 16 hours instead of at 100°C for 4 hours.

Method 5. This was essentially the KCl method proposed by Øien and Selmer-Olsen[16]. In the procedure used, 4.0 g of soil were placed in a 60-ml bottle and treated with 40 ml of 2M KCl. The bottle was sealed with a rubber stopper and placed for 20

hours in a water bath maintained at 80°C. The bottle was then removed from the water bath, cooled to room temperature, and shaken manually for 10 to 15 sec. The soil-KCl suspension was filtered through a Whatman no. 42 filter paper, and the filtrate was analysed for ammonium-N by the steam distillation procedure described by Bremner and Keeney[17]. The ammonium-N present in the soil sample before heating with 2M KCl was determined by the method of Bremner and Keeney[17], and the ammonium-N produced by heating with 2M KCl was calculated as the difference between the results of these two analyses.

Method 6. This was essentially the method proposed by Whitehead[18]. In the procedure used, 3.0 g of soil were treated with 20 ml of 1M KCl in a Pyrex digestion tube (25 mm x 300 mm), and the mixture was boiled gently for 1 hour in a Tecator block digester. The ammonium-N present after this treatment was determined by steam distillation of the soil-KCl mixture with MgO as described in method 3. The ammonium-N present before the treatment with 1M KCl was also determined as described in method 3, and the ammonium-N produced by this treatment was calculated as the difference between the results of these two analyses.

Method 7. This was a modification of method 6 in which the soil-KCl mixture was heated in a stoppered tube at 100°C for 1 hour instead of being boiled in an unstoppered tube at 100°C for 1 hour.

Method 8. This was the phosphate-borate buffer method under evaluation. It involves determination of the ammonia-N produced from organic soil N when soil is steam-distilled with phosphate-borate buffer. In this method, 4.0 g of soil are steam-distilled with 40 ml of pH 11.2 phosphate-borate buffer for 8 min, and the ammonia-N released by this distillation is determined. The ammonium-N initially present in the soil sample is determined by estimating the ammonia-N liberated by steam-distilling 4.0 g of soil with 0.2 g of MgO and 20 ml of 2M KCl for 3.3 min, and the ammonium-N produced from organic soil N by heating with pH 11.2 buffer is calculated as the difference between the results of these two analyses.

Method 9. This was the $CaCl_2$-autoclave method recommended in the recent revision of the ASA monograph on "Methods of Soil Analysis"[4]. In the procedure adopted, 10 g of soil were treated with 25 ml of 0.01M $CaCl_2$ in a 50-ml Pyrex test tube, and the tube was capped with Al foil and placed in an autoclave at 121°C for 16 hours. The tube and its contents were allowed to cool to room temperature, and the soil-$CaCl_2$ mixture was transferred to a 200-ml distillation flask designed for use with the steam distillation apparatus described by Bremner[19], the transfer being completed by rinsing the tube with 25 ml of 4M KCl. The ammonium-N present in the soil-$CaCl_2$-KCl mixture was then determined by steam distillation of the mixture with 0.2 g of MgO for 3.3 min as described by Keeney and Bremner[20]. The ammonium-N present in the soil before autoclaving was determined by the steam distillation method described by Keeney and Bremner[20], and the ammonium-N produced by autoclaving was calculated as the difference between the results of these two analyses.

Method 10. This was essentially the acid permanganate method proposed by Stanford and Smith[9]. In the procedure used, 1.0 g of soil was placed in a 50-ml plastic centrifuge tube and treated with 25 ml of 1N $H_2SO_4$. The tube was then stoppered, shaken for 1 hour, and centrifuged. The supernatant liquid was discarded, the soil residue was treated with 25 ml of 0.05N $KMnO_4$:1.0N $H_2SO_4$ solution, and the tube was again stoppered, shaken for 1 hour, and centrifuged. Ammonium-N in the supernatant liquid was determined by steam distillation of an aliquot with 10N NaOH.

Method 11. This was essentially an alkaline permanganate method described by Stanford[10]. In the procedure followed, 1.0 g of soil was placed in a 100-ml distillation flask designed for use with the steam distillation apparatus described by Bremner[19] and treated with 10 ml of 0.25N NaOH containing 0.1 g of $KMnO_4$, and the $NH_3$-N released by steam distillation of this mixture for 4 min was determined as described by Bremner[19]. The $NH_3$-N released by steam distillation of 1.0 g of soil with 0.25N NaOH (10 ml) for 4 min in the absence of $KMnO_4$ was also determined, and the $NH_3$-N

produced by $KMnO_4$ oxidation was calculated as the difference between the results of these two analyses.

Method 12. This was the $NaHCO_3$ UV method proposed by Fox and Piekielek[11]. In this method, 5.0 g of soil were shaken with 100 ml of 0.01M $NaHCO_3$ for 15 min, and the resulting suspension was filtered through Whatman no. 42 filter paper. The absorption of the filtrate at 260 nm was then measured with a Model DB-GT Beckman spectrophotometer using matched quartz 1-cm cells.

Biological Methods

Method 13. This was the waterlogged incubation method of Waring and Bremner[21] modified as described by Keeney and Bremner[22]. It involved determination of the ammonium-N produced when 5 g of soil were incubated under waterlogged conditions at 40°C for 7 days.

Method 14. This was the aerobic incubation method described by Keeney and Bremner[23]. It involved determination of the ($NH_4^+$ + $NO_3^-$ + $NO_2^-$)-N produced when 10 g of soil were mixed with 30 g of 30- to 60-mesh quartz sand, moistened with 6 ml of distilled water, and incubated under aerobic conditions at 30°C for 14 days.

Method 15. This was a modification of the aerobic incubation method suggested by Stanford and Smith[12], the modification being that the time of incubation was reduced from 30 to 12 weeks[4,12]. In the procedure used, 15 g of soil were thoroughly mixed with 15 g of water-washed quartz sand (30-60 mesh) and placed in a 100-ml leaching tube fitted at its base with a plug of glass wool. The surface of the soil-sand mixture in the tube was then covered with a thin layer of glass wool, and mineral N [($NH_4^+$ + $NO_3^-$ + $NO_2^-$)-N] in the mixture was removed by leaching with 100 ml of 0.01M $CaCl_2$ in 5- to 10-ml increments, followed by 25 ml of a N-free nutrient solution [0.002M $CaSO_4.2H_2O$:0.002M $MgSO_4$:0.005M $Ca(H_2PO_4)_2.H_2O$:0.0025M $K_2SO_4$]. The moisture content of the soil-sand mixture was adjusted as described by Stanford and Smith[12], and the tube was covered by a piece of plastic (Saran) film with a hole (0.5 cm in diameter) in the center and placed in an incubator

maintained at 35°C. Mineral N produced by incubation of the soil-sand mixture at this temperature was removed by leaching after 2, 4, 6, 8, and 12 weeks, leaching being performed with 100 ml of 0.01M $CaCl_2$ in 5- to 10-ml increments followed by 25 ml of a N-free nutrient solution. After each leaching, the moisture content of the soil-sand mixture was adjusted as described by Stanford and Smith[12]. Mineral N in the leachates was determined by the steam distillation method of Bremner and Keeney[17].

Method 16. This was a modification of the method 15 in which only the mineral N produced by incubation of the soil-sand mixture for 2 weeks was determined.

Method 17. This involved calculation of the soil N mineralization potential value ($N_o$) as defined by Stanford and Smith[12] from the mineralizable N data obtained by method 15. In the procedure used, a Statistical Analysis System (SAS) computerized non-linear least squares technique utilizing Marquardt's method (Barr et al.[24]) was used to fit the mineralizable N data obtained by method 15 to the first order equation $Nt = N_o(1-e^{-kt})$, where Nt = μg mineral N produced per g of soil in time t (weeks), $N_o$ = N mineralization potential value expressed as μg N $g^{-1}$ soil, and k = N mineralization rate constant (in $weeks^{-1}$) [25,26]. Initial values for both $N_o$ and k were calculated from the N mineralized between 2 and 12 weeks. Final $N_o$ values were computed by adding the N mineralized during the 0- to 2-week period of incubation to the initial $N_o$ values because Stanford and Smith[12] found that this procedure provided the most reliable estimate of $N_o$ (see also Griffin and Laine[26]). Unless otherwise specified, all analyses reported were performed in triplicate or quadruplicate. Statistical analyses were performed using the Statistical Analysis System (SAS)[24].

## RESULTS AND DISCUSSION

Table 2 shows the measurements performed in the 12 chemical methods and 5 biological methods used to obtain an index of potentially available organic N in the 30 soils studied. The

TABLE 2

Methods Used to Obtain an Index of Potentially Available Organic N in Soil

| Method | Measurement performed |
|---|---|
| | Chemical methods |
| 1 | Organic C |
| 2 | Total N |
| 3 | $NH_4^+$-N produced by heating soil with 2M KCl at 100°C for 4 hours |
| 4 | $NH_4^+$-N produced by heating soil with 2M KCl at 95°C for 16 hours |
| 5 | $NH_4^+$-N produced by heating soil with 2M KCl at 80°C for 20 hours |
| 6 | $NH_4^+$-N produced by boiling soil with 1M KCl for 1 hour in unstoppered tube |
| 7 | $NH_4^+$-N produced by heating (100°C) soil with 1M KCl for 1 hour in stoppered tube |
| 8 | $NH_3$-N released by steam distillation of soil with pH 11.2 phosphate-borate buffer solution |
| 9 | $NH_4^+$-N released on autoclaving soil treated with 0.01M $CaCl_2$ |
| 10 | $NH_4^+$-N extracted from soil by acidic permanganate solution |
| 11 | $NH_3$-N produced by steam distillation of soil with alkaline permanganate solution |
| 12 | Ultraviolet absorbance (260 nm) of $NaHCO_3$ extract of soil |
| | Biological methods |
| 13 | $NH_4^+$-N produced on anaerobic incubation of soil at 40°C for 1 week |
| 14 | $(NH_4^+ + NO_3^- + NO_2^-)$-N produced on aerobic incubation of soil at 30°C for 2 weeks |
| 15 | $(NH_4^+ + NO_3^- + NO_2^-)$-N produced on aerobic incubation of soil at 35°C for 12 weeks |
| 16 | $(NH_4^+ + NO_3^- + NO_2^-)$-N produced on aerobic incubation of soil at 35°C for 2 weeks |
| 17 | Nitrogen mineralization potential ($N_o$) |

results obtained by the chemical methods are reported in Tables 1, 3, and 4, and the results obtained by the biological methods are reported in Table 5. The correlation coefficients for the relationships between the results of the various chemical and biological methods used are reported in Table 6, and the correlation coefficients for the relationships between the results of the biological methods used are reported in Table 7.

The precisions of the chemical methods used were compared by performing 6-8 analyses by each method on the Storden, Marshall, and Okoboji soils. The average coefficients of variation of the results of these analyses were as follows: method 1, 1.5%; method 2, 0.9%; method 3, 2.1%; method 4, 1.5%; method 5, 3.0%; method 6, 3.3%; method 7, 3.1%; method 8, 2.0%; method 9, 3.0%; method 10, 3.3%; method 11, 3.8%; method 12, 3.5%.

Methods 1 and 2 (organic C and total N)

Organic soil C (method 1) and total soil N (method 2) were included as indexes of potentially available organic soil N because several workers have found that the results of biological and chemical methods of assessing soil N availability are closely related to total soil N, which in turn is closely related to organic soil C or soil organic-matter content, and several states are using soil organic-matter content as a basis for N fertilizer recommendations (see Keeney[4]). Table 6 shows that the results obtained by method 1 (organic soil C) and method 2 (total soil N) were significantly related to those obtained by the biological methods but that the correlation coefficients for these relationships were lower than those obtained with most of the other chemical methods used. Stanford and Smith[9] concluded from work with 62 soils that total soil N is a poor index of soil N availability, and the data reported in Table 6 support this conclusion. It is noteworthy, however, that the correlation coefficient we obtained for the relationship between total soil N and $N_o$ (Table 6) was higher than that observed in previous work by Stanford and Smith[9] and Griffin and Laine[26]. As noted by Keeney[4], determination of total N in soil by the Kjeldahl method is too

TABLE 3

Nitrogen Values Obtained by Chemical Methods 3-7

| Soil | Method 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|
| | μg N $g^{-1}$ soil | | | | |
| Thurman | 5.2 | 6.9 | 3.2 | 2.2 | 2.9 |
| Storden | 5.3 | 5.8 | 2.9 | 0.5 | 0.9 |
| Dickinson | 7.4 | 9.0 | 4.9 | 2.1 | 3.8 |
| Buckney | 5.2 | 8.9 | 4.9 | 1.2 | 4.0 |
| Sparta | 11.0 | 14.3 | 5.7 | 4.0 | 4.2 |
| Ida | 12.4 | 18.4 | 7.4 | 5.9 | 6.3 |
| Dickinson | 13.7 | 26.4 | 9.9 | 7.0 | 7.8 |
| Clarinda | 17.4 | 19.1 | 6.9 | 4.1 | 7.8 |
| Clarion | 14.0 | 19.7 | 10.1 | 5.5 | 7.1 |
| Monona | 14.5 | 20.7 | 9.2 | 5.4 | 5.5 |
| Shelby | 16.6 | 21.7 | 9.5 | 5.1 | 7.9 |
| Fayette | 21.6 | 30.3 | 13.5 | 7.1 | 8.1 |
| Hayden | 26.9 | 35.5 | 18.7 | 7.2 | 9.1 |
| Nicollet | 15.6 | 22.4 | 7.4 | 5.5 | 5.7 |
| Muscatine | 29.5 | 38.4 | 18.3 | 8.6 | 10.3 |
| Marshall | 20.5 | 37.6 | 16.5 | 9.4 | 9.6 |
| Grundy | 16.4 | 24.4 | 9.2 | 5.3 | 6.1 |
| Readlyn | 21.6 | 27.0 | 13.3 | 6.3 | 7.1 |
| Wacousta | 23.1 | 32.0 | 12.7 | 6.3 | 7.4 |
| Webster | 21.0 | 34.2 | 18.7 | 8.9 | 9.9 |
| Galva | 20.6 | 30.0 | 13.2 | 4.6 | 5.3 |
| Webster | 14.8 | 28.0 | 12.3 | 3.7 | 4.6 |
| Okoboji | 20.0 | 23.2 | 8.6 | 1.3 | 1.9 |
| Webster | 24.2 | 36.0 | 16.8 | 8.4 | 8.9 |
| Wacousta | 26.8 | 46.3 | 15.5 | 8.3 | 9.2 |
| Weller | 46.7 | 67.5 | 31.3 | 11.9 | 13.9 |
| Canisteo | 18.2 | 31.2 | 8.7 | 0.5 | 1.0 |
| Canisteo | 31.7 | 51.4 | 17.9 | 5.8 | 8.5 |
| Harps | 30.0 | 53.3 | 26.1 | 6.0 | 8.2 |
| Tillamook | 48.9 | 81.0 | 33.6 | 18.2 | 24.2 |
| Average | 20.0 | 30.0 | 12.9 | 5.9 | 7.2 |

TABLE 4

Values Obtained by Chemical Methods 8-12

| Soil | Method 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|
| | ---------- μg N $g^{-1}$ soil ---------- | | | | Absorbance |
| Thurman | 11.1 | 20 | 38 | 39 | 0.62 |
| Storden | 11.9 | 8 | 31 | 32 | 0.32 |
| Dickinson | 13.3 | 27 | 48 | 63 | 0.50 |
| Buckney | 9.7 | 19 | 21 | 49 | 0.52 |
| Sparta | 21.6 | 40 | 72 | 86 | 0.76 |
| Ida | 18.9 | 30 | 51 | 15 | 0.45 |
| Dickinson | 34.8 | 69 | 73 | 132 | 1.04 |
| Clarinda | 36.7 | 70 | 77 | 153 | 0.90 |
| Clarion | 35.3 | 50 | 79 | 130 | 0.79 |
| Monona | 14.0 | 54 | 87 | 154 | 0.86 |
| Shelby | 53.4 | 85 | 97 | 154 | 0.86 |
| Fayette | 42.6 | 86 | 78 | 88 | 0.61 |
| Hayden | 45.6 | 92 | 141 | 157 | 0.77 |
| Nicollet | 35.1 | 50 | 68 | 140 | 0.86 |
| Muscatine | 56.5 | 110 | 133 | 130 | 1.02 |
| Marshall | 57.7 | 114 | 107 | 172 | 1.13 |
| Grundy | 42.7 | 73 | 94 | 150 | 0.82 |
| Readlyn | 39.6 | 81 | 73 | 49 | 0.55 |
| Wacousta | 42.5 | 84 | 124 | 161 | 0.92 |
| Webster | 41.2 | 80 | 100 | 147 | 0.68 |
| Galva | 62.2 | 90 | 107 | 148 | 0.99 |
| Webster | 32.8 | 80 | 83 | 38 | 0.51 |
| Okoboji | 31.2 | 66 | 108 | 135 | 0.56 |
| Webster | 41.2 | 116 | 96 | 57 | 0.96 |
| Wacousta | 60.1 | 143 | 136 | 124 | 1.20 |
| Weller | 97.8 | 158 | 157 | 120 | 1.74 |
| Canisteo | 31.9 | 72 | 90 | 150 | 0.64 |
| Canisteo | 69.9 | 122 | 104 | 141 | 0.74 |
| Harps | 54.8 | 154 | 100 | 112 | 0.69 |
| Tillamook | 99.6 | 129 | 305 | 151 | 1.18 |
| Average | 41.5 | 79 | 96 | 113 | 0.81 |

TABLE 5

Nitrogen Values Obtained by Biological Methods

| Soil | Method 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|
| | ---------- μg N $g^{-1}$ soil ---------- | | | | |
| Thurman | 10 | 5 | 21 | 7 | 26 |
| Storden | 14 | 5 | 27 | 8 | 31 |
| Dickinson | 22 | 14 | 30 | 9 | 40 |
| Buckney | 27 | 12 | 26 | 8 | 35 |
| Sparta | 27 | 19 | 59 | 19 | 77 |
| Ida | 50 | 15 | 77 | 32 | 105 |
| Dickinson | 56 | 35 | 78 | 17 | 101 |
| Clarinda | 34 | 28 | 115 | 37 | 122 |
| Clarion | 48 | 19 | 72 | 19 | 105 |
| Monona | 58 | 25 | 89 | 26 | 99 |
| Shelby | 77 | 35 | 137 | 38 | 163 |
| Fayette | 75 | 44 | 99 | 30 | 132 |
| Hayden | 104 | 62 | 167 | 57 | 207 |
| Nicollet | 58 | 19 | 69 | 24 | 86 |
| Muscatine | 94 | 51 | 166 | 62 | 202 |
| Marshall | 95 | 56 | 191 | 52 | 231 |
| Grundy | 55 | 15 | 103 | 28 | 124 |
| Readlyn | 66 | 26 | 95 | 40 | 121 |
| Wacousta | 89 | 44 | 132 | 43 | 162 |
| Webster | 61 | 9 | 96 | 27 | 160 |
| Galva | 71 | 25 | 156 | 40 | 200 |
| Webster | 48 | 22 | 57 | 17 | 110 |
| Okoboji | 51 | 24 | 88 | 29 | 107 |
| Webster | 73 | 45 | 127 | 54 | 186 |
| Wacousta | 101 | 36 | 176 | 49 | 249 |
| Weller | 145 | 171 | 291 | 138 | 320 |
| Canisteo | 45 | 28 | 99 | 34 | 115 |
| Canisteo | 126 | 57 | 216 | 81 | 284 |
| Harps | 92 | 39 | 134 | 41 | 259 |
| Tillamook | 161 | 135 | 349 | 124 | 383 |
| Average | 68 | 37 | 118 | 40 | 151 |

TABLE 6

Correlation Coefficients for the Relationships Between the Results of Chemical and Biological Methods

| Chemical method | Biological methods | | | | |
|---|---|---|---|---|---|
| | 13 | 14 | 15 | 16 | 17 |
| | ---------- Correlation coefficient (r)* ---------- | | | | |
| 1 | 0.75 | 0.62 | 0.77 | 0.71 | 0.82 |
| 2 | 0.79 | 0.65 | 0.80 | 0.73 | 0.86 |
| 3 | 0.95 | 0.88 | 0.94 | 0.94 | 0.95 |
| 4 | 0.94 | 0.84 | 0.93 | 0.90 | 0.96 |
| 5 | 0.91 | 0.84 | 0.88 | 0.87 | 0.93 |
| 6 | 0.83 | 0.76 | 0.83 | 0.79 | 0.81 |
| 7 | 0.83 | 0.79 | 0.85 | 0.81 | 0.83 |
| 8 | 0.93 | 0.84 | 0.95 | 0.91 | 0.95 |
| 9 | 0.87 | 0.70 | 0.82 | 0.77 | 0.92 |
| 10 | 0.84 | 0.77 | 0.89 | 0.82 | 0.85 |
| 11 | 0.48 | 0.31 | 0.52 | 0.36 | 0.48 |
| 12 | 0.69 | 0.76 | 0.76 | 0.74 | 0.69 |

*r-values between 0.47 and 0.61 are significant at 1% level; r-values above 0.61 are significant at 0.1% level.

TABLE 7

Correlation Coefficients for the Relationships Between the Results of Biological Methods

| Method | Method | | | |
|---|---|---|---|---|
| | 14 | 15 | 16 | 17 |
| | ----- Correlation coefficient (r)* ----- | | | |
| 13 | 0.85 | 0.95 | 0.91 | 0.96 |
| 14 | | 0.89 | 0.95 | 0.81 |
| 15 | | | 0.96 | 0.96 |
| 16 | | | | 0.90 |

*All r-values reported are significant at 0.1% level.

costly an analysis for soil testing laboratories, whereas organic C or organic matter in soil can be readily determined by indirect $K_2Cr_2O_7$ oxidation methods. However, Table 6 shows that, although the results of analyses for organic C (method 1) were significantly related to the results of the biological methods, the correlation coefficients for these relationships were much lower than those obtained with other chemical methods that are simpler and more rapid (e.g., methods 3 and 8).

Methods 3-7 (KCl methods)

Of the five methods tested that involved determination of the $NH_4^+$-N produced by heating soil samples with 1M or 2M KCl (methods 3-7), those involving heating with 2M KCl at 100°C for 4 hours (method 3) or at 95°C for 16 hours (method 4) gave the highest correlations when their results were compared with those obtained by the biological methods (Table 6). The results obtained by methods 3 and 4 accounted for 90% and 92%, respectively, of the variation in the N mineralization potential ($N_o$) values of the 30 Iowa soils studied. It is concluded that the KCl procedure developed in our laboratory for rapid assessment of potentially available organic N in Brazilian soils (method 3) is also satisfactory for Iowa soils and that this procedure merits consideration for use in soil testing laboratories. Method 4 gave results that were almost as closely related to those obtained by the four incubation methods (13-16) as were the results obtained by method 3 (Table 6), and it was slightly more precise than method 3. It has the disadvantage, however, of requiring a 16-hour period of heating.

Method 5 was essentially the KCl method proposed by Øien and Selmer-Olsen[16], the only significant difference being that the $NH_4^+$-N produced by heating the soil sample with 2M KCl in a stoppered tube in a water bath maintained at 80°C for 20 hours was determined by a steam distillation procedure instead of by a colorimetric method. Table 6 shows that the results obtained by this method were closely related to those obtained by the biological methods. The disadvantages of method 5 compared with

methods 3 and 4 are that it requires a 20-hour period of heating and a filtration step. It is also less precise than method 3 or 4, presumably because the amount of $NH_4^+$-N produced in method 5 is considerably smaller than the amount produced in method 3 or 4 (Table 3).

Method 6 was essentially the KCl procedure proposed by Whitehead[18], the only difference being that the $NH_4^+$-N produced by boiling the soil sample with 1M KCl for 1 hour was determined by direct distillation of the soil-KCl mixture with MgO instead of by filtering this mixture and analyzing an aliquot of the extract by a colorimetric procedure. The correlation coefficients reported in Table 6 show that this method and method 7 were not as good as the other KCl methods evaluated (methods 3-5) when they were compared with biological methods. Method 6 was also the least precise of the five KCl methods studied.

Method 7 was a modification of method 6 in which the soil sample was heated with 1M KCl at 100°C in a stoppered tube for 1 hour instead of being boiled with 1M KCl in an unstoppered tube for 1 hour. This method was studied because it seemed likely that $NH_3$ is lost when soil is boiled with 1M KCl as in method 6 and that this is at least partly responsible for the poor precision of this method. These conclusions were supported by our finding that the $NH_4^+$-N values obtained by method 7 were higher than those obtained by method 6 (Table 3) and that method 7 gave better results than method 6 (Table 6) and was considerably more precise.

Method 8 (phosphate-borate buffer method)

The results obtained by this method were highly correlated with those obtained by the biological methods (Table 6), and they accounted for approximately 90% of the variation in the N mineralization potential ($N_o$) values of the 30 soils studied. This supports our conclusion from previous work[6,7] that this rapid steam distillation method merits consideration for use in soil testing laboratories.

Method 9 (calcium chloride-autoclave method)

Table 6 shows that, although the results obtained by this chemical method were significantly related to those obtained by the five biological methods, the correlation coefficients for these relationships were lower than those obtained with chemical methods 3, 4, and 8, which are simple and rapid by comparison. The $CaCl_2$-autoclave method evaluated was the modification of the Stanford and Smith[27] method recommended in the recent revision of the ASA monograph on "Methods of Soil Analysis"[4]. This modified method is much simpler than the original procedure, but it is not simple enough for use in soil testing laboratories because considerable care is needed in the transfer of the autoclaved soil-$CaCl_2$ mixture for ammonium analysis (see also Magdoff et al.[28]).

Method 10 (acid permanganate method)

Table 6 shows that the results obtained by this chemical method were significantly related to those obtained by the biological methods, but that the correlation coefficients for these relationships were lower then those obtained with other chemical methods (e.g., methods 3, 4, 5, and 8). The acid permanganate method evaluated was the procedure described by Stanford and Smith[9], which involves extraction of the soil sample with acid $KMnO_4$ solution after extraction with 1N $H_2SO_4$, the 1N $H_2SO_4$ extract being discarded. It is not suited for use in soil testing laboratories because it requires two extractions and two centrifugations. Moreover, we found that the amount of $NH_4^+$-N extracted by 1N $H_2SO_4$ was more than twice the amount extracted with 2M KCl, which means that the treatment with 1N $H_2SO_4$ extracted organic N that was readily hydrolyzed to $NH_4^+$-N and was not recovered by the subsequent extraction with acid $KMnO_4$ solution. Stanford and Smith[9] concluded that this method was superior to the $CaCl_2$-autoclave method because it is suitable for both calcareous and noncalcareous soils, whereas the $CaCl_2$-autoclave method is not suitable for certain calcareous soils. Table 6 shows that the acid $KMnO_4$ method (method 10) was superior

to the $CaCl_2$-autoclave method (method 9) when the results of these two chemical methods were compared with those obtained by the biological methods 14, 15, and 16 but that the $CaCl_2$-autoclave method (method 9) was superior to the acid $KMnO_4$ method (method 10) when the results of these methods were compared with those obtained by the biological methods 13 and 17 with 30 Iowa soils that included 8 calcareous soils.

Method 11 (alkaline permanganate method)

Although a variety of alkaline permanganate methods have been proposed for assessment of potentially available organic N in soil, most workers have found that these methods do not give satisfactory results (e.g., Stanford[10], Keeney and Bremner[22], Olson et al.[29], Jenkinson[30], Cornforth and Walmsey[31]). Table 6 shows that, of the 12 chemical methods evaluated in our work, the alkaline permanganate method (method 11) gave the lowest correlation coefficients when its results were compared with those obtained by the biological methods. Method 11 also had the poorest precision of the 12 chemical methods studied.

Method 12 ($NaHCO_3$ UV method)

Table 6 shows that, although the results obtained by this chemical method were significantly related to those obtained by the biological methods, the correlation coefficients for these relationships were considerably smaller than those obtained with most of the other chemical methods studied. Other workers have found that the results of this method are not closely related to those obtained by incubation methods or to N uptake by plants in greenhouse pot experiments (e.g., Rodrigues Filho[32], Whitehead et al.[33], Fox and Piekielek[34]). This method would not be satisfactory for use in soil testing laboratories even if it provided a good index of soil N availability because it requires filtration of the suspension obtained by shaking the soil sample with 0.01M $NaHCO_3$, and it is usually necessary to centrifuge this suspension to facilitate filtration.

## Conclusions

To conclude, the work reported showed that the results obtained with 30 Iowa soils by the two rapid and simple chemical methods of assessing potentially available organic soil N under evaluation (methods 3 and 8) were highly correlated with the results of the five biological methods used for comparison and that the correlations observed with these two methods were higher than those obtained with chemical methods previously proposed. Both methods also have important advantages over previous chemical methods in that they do not involve filtration or transfer steps and are well suited for use in soil testing laboratories. It should be emphasized, however, that, like previous chemical methods, these two methods provide only an index of potentially available organic soil N and that inorganic soil N and factors such as climate and management must be taken into consideration when these methods are used to aid prediction of N fertilizer requirements[4]. It is noteworthy in this connection that the steam distillation apparatus and several of the reagents employed in these methods can also be used for determination of inorganic N in soil by rapid steam distillation methods[17,20].

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