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COMPARISON OF DIFFERENT PROCEDURES FOR THE DETERMINATION OF AMMONIUM IN SOIL EXTRACTS

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การเปรียบเทียบวิธีการวัดแอมโมเนียมในสารละลายที่สกัดได้จากดินโดยวิธีต่างๆ

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บทคัดย่อ : การทดลองในห้องปฏิบัติการได้ทำขึ้นเพื่อเปรียบเทียบวิธีการ 3 วิธี ที่ใช้ในการหาความเข้มข้นของแอมโมเนียมในดินโดยใช้ 0.01 M $CaCl_2$ เป็นตัวสกัด วิธีการ 3 วิธีดังกล่าวคือ : NH_3-gas diffusion electrode, Continuous flow system (CFS) และ High Pressure Liquid Chromatography (HPLC) อิทธิพลของระยะเวลาที่ปล่อยสารละลายทิ้งไว้ในช่วงระยะเวลาหนึ่งก็ได้รับการทดสอบด้วย ความเข้มข้นของแอมโมเนียมในสารละลายที่สกัดด้วย 0.01 M $CaCl_2$ และวัดโดยวิธี CFS และวิธี HPLC ให้ค่าใกล้เคียงมาก ค่าที่ได้จากการวัดโดยวิธี Gas-diffusion electrode ต่ำกว่าค่าที่วัดโดย CFS เล็กน้อยในตัวอย่างดินที่อบที่อุณหภูมิ 40 °C แต่สำหรับตัวอย่างดินที่อบที่อุณหภูมิ 105 °C ค่าที่ได้จากการวัดโดยวิธี Gas-diffusion electrode จะสูงกว่าค่าที่วัดโดย CFS เล็กน้อย อย่างไรก็ตาม เมื่อคำนึงถึงปัญหาเกี่ยวกับการวิเคราะห์ค่าความเข้มข้นที่ต่ำของแอมโมเนียมโดยใช้ Gas-diffusion electrode แล้วจึงสรุปว่าค่าดังกล่าวไม่แตกต่างกันในทางสถิติ ค่าความเข้มข้นของแอมโมเนียมในสารละลายที่ได้จากดินไม่เปลี่ยนแปลง ในช่วงระยะเวลาที่เก็บไว้อย่างน้อย 5 ชั่วโมง ผลการทดลองนี้ให้ผลเหมือนกันถ้าใช้เวลาในการเขย่าดินเพียง 30 นาที แทนที่จะเป็น 2 ชั่วโมงดังปกติ.

ABSTRACT : A laboratory experiment was set up to determine the ammonium concentration in 0.01 M $CaCl_2$ soil extracts by three different procedures : an NH_3 - gas diffusion electrode, a Continuous Flow System (CFS) and High Pressure Liquid Chromatography (HPLC). The influence of time on the measured ammonium concentration when leaving the extracting solutions to stand was also tested. The NH^+_4-nitrogen concentration in soil samples extracted with 0.01 M $CaCl_2$ and measured by a continuous-flow technique and HPLC gave almost equal values. The values obtained by measurement with a gas-diffusion electrode was slightly lower than those obtained by CFS in the soil samples dried at 40 °C but slightly higher than the samples dried at 105 °C. The analytical problems in the measurement of low NH^+_4-nitrogen concentration using a gas - diffusion electrode is that these values do not differ statistically. The NH^+_4-nitrogen concentration

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in the soil extracts does not change during storage for at least 5 hours. The same result was found when this soil was shaken for only 30 minutes instead of the conventional 2 hours. The concentration of measured NH^+_4 was not influence by shaking time and standing time.

INTRODUCTION

The determination of NH^+_4 in soil samples is still problematic. The results of the NH^+_4 determination are varied due to analytical procedures. Moreover, the conditions such as drying temperature of the soil and time of measurement after extraction may influence the results. It was assumed that when soil extracts are measured at high pH more NH^+_4 might be found due to hydrolysis of organic nitrogen in the extracts during measurement. The study of Bogdanovic (1989) proved that the ammonium concentration was higher at high drying temperature (105 °C) than at low drying temperature (40 °C). She concluded also that the ammonium concentration was higher when using a continuous flow system (spectrophotometric, CFS) than using a chromatographic detection procedure. Villalobos (1989) reported that 0.01 M $CaCl_2$ soil extracts could be analyzed for ammonium concentration by using the ammonium gas-selective electrode when the concentrations is higher than 3×10^{-6} M which equals 0.42 mg NH^+_4-nitrogen per kg dry soil.

The aim of this study is to compare the ammonium concentration in 0.01 M $CaCl_2$ soil extracts by 3 different procedures : an ammonium-gas diffusion electrode, a CFS and high pressure liquid chromatography (HPLC). The influence of time the measured NH^+_4 concentration on stand leaving the extracting solutions was also tested.

MATERIALS AND METHODS

Soil samples

Six soil types in the surrounding of Wageningen, The Netherlands, were selected. The soil types differed in chemical and physical properties. The name of the soil types are as follows:

Veenendaal : a peat soil, Uiterwaarden : a calcareous river clay soil, Droevendaal : a sandy soil, Engsoil : a sandy soil rich in phosphate, Nude soil : a river clay soil, and Heide : a poor sandy soil.

After mixing, each soil sample was divided into two parts : first part was dry at 40 °C (with ventilation) and the other part at 105 °C. Thereafter the dry soil samples were throughly mixed and ground to the fraction < 2 mm.

Extraction

Eight g of each soil sample was weighted out and placed into 100 ml centrifuge tubes. Then 80 ml of 0.01 M $CaCl_2$ was added. After adding the extracting solution, the soil samples were shaken immediatedly at room temperature for 2 hours. After shaking, the centrifuge tubes were centrifuged for 10 minutes at 2000-2200 rpm.

Procedure

Clear supernatant were used for the determination of ammonium by :

Measurement of NH^+_4 with an NH_3-gas diffusion electrode

1. The standard series had NH_3 concentration of 10^{-6}, $3x10^{-6}$, 10^{-5}, $3x10^{-5}$, $3x10^{-4}$ and $1x10^{-3}$ M.
2. 50 ml of the standard series as well as the clear supernatant (soil extracts) were used for the measurement.
3. The electrical potential for each sample was recorded at 0.5 minute intervals during a period of 5 minutes. Each standard solution and soil extracts was measured at least in duplicate. Since the readings were not steady, the values were chosen when the variation of potential was 1 mv/30 seconds, both for the standard series and soil extracts.

Measurement of NH^+_4 with a CFS of ammonium were done by Mr. Heij, Department of Soil Science and Plant Nutrition, Wageningen Agricultural University, The Netherlands. Briefly procedures are as follows: NH^+_4 was determined spectrophotometrically with the Berthelot reaction, in which a phenol derivative formed and azodye in the presence of hypochlorite and salicylate. The extinction of the formed indophenol blue complex was measured at 660 nm and was in relation to the concentration of the ammonia.

Measurements of NH^+_4 with HPLC were done by Department of microbiology, Wageningen Agricultural University, The Netherlands. Principles of the measurement are as given by Hasset (1982).

RESULTS AND DISCUSSION

Ammonium concentration measured by ammonium gas-selective electrode and CFS.

Six soil samples were extracted in duplicate. 50 ml of each clear extract was pipetted to be measured with the ammonium gas-selective electrode. From the same extracts, another 15 ml was pipetted and analysed by using CFS. The results from ammonium gas-selective electrode and CFS methods are given in Table 1.

The results from Table 1 (40 °C) show that most of the values obtained with CFS are higher than the values obtained with the ammonium gas-selective electrode.

The results from Table 1 (150 °C) show that, however, most of the values obtained with the ammonium gas-selective electrode are higher than that obtained with CFS except for the Droevendaal soil.

Table 1. NH^+_4-nitrogen concentration in soil samples extracted with 0.01 M $CaCl_2$ soil extracts as measured by an ammonium gas-selective electrode and CFS.

Soil samples	NH^+_4-nitrogen conc. (mg N/kg oven-dry soil)			
	Gas electrode		CFS	
	Replication 1	Replication 2	Replication 1	Replication 2
Dried at 40 °C				
Veen.	1.24	1.25	1.96	2.06
Uiterw.	1.54	1.68	1.94	2.55
Droeven.	2.21	2.18	2.41	2.41
Eng.	4.62	5.23	5.86	5.56
Nude.	3.39	3.18	3.26	3.37
Heide	5.24	5.42	5.83	5.83
Dried at 105 °C				
Veen.	16.59	16.44	13.55	13.45
Uiiterw.	17.60	18.98	15.76	16.46
Droeven.	5.33	5.89	6.11	6.11
Eng.	12.90	12.56	11.72	11.52
Nude.	12.09	11.95	10.94	10.94
Heide	10.38	11.25	10.32	10.62

Influence of time on the ammonium concentration

The Heide soil dried at 40 °C was chosen for this study. Eleven sub samples of this soil were extracted and the shaking time was 2 hours. After centrifuging, the soil extracts were measured at different time intervals using the gas-diffusion electrode according to the procedure described before. The initial measurement was made at 15 minutes after centrifuging and there after using 30 minutes time interval. The results from Table 2 show that there is hardly any influence of time (up to about 5 hours) on the ammonium concentration in the soil extracts. Therefore, it can be concluded that the soil extracts can be stored at room temperature up to approximately 5 hours (after extraction) without any change in the ammonium concentration.

Table 2. Influence of time on the ammonium concentration in soil extracts. (Shaking time 2 hours).

Time after contrifuging (min.)	NH^+_4 concentration mg N/kg oven dry soil
0	5.21
15	5.35
45	4.65
75	5.42
105	4.86
135	4.76
165	5.17
195	4.96
225	5.18
255	5.46
285	5.63

Influence of time and shaking time on the ammonium concentration.

The Heide soil dried at 40 °C and 105 °C were used. Eleven sub samples for each drying temperature were extracted. The shaking time was 30 minutes instead of 2 hours. After centrifuging, the clear supernatants for all soil samples were pipetted into clean vessels and then well closed with a covers. The first measurement was made at 15, and thereafter 30 minutes time interval. The results are given in Table 3.

It clearly show that there is no influence of shaking time (2 hours) and standing time on the measure NH^+_4-nitrogen concentration in the Heide soil (Table 2 and 3).

Table 3. Influence of shaking time and waiting on the ammonium concentration (Shaking time 30 minutes).

Time after centrifuging (min.)	NH^+_4 conc. (mg N/kg soil)	
	Heide 40 °C	Heide 105 °C
0	4.93	9.67
15	4.94	10.94
30	4.85	10.24
60	4.94	11.22
90	5.00	10.66
120	5.49	11.36
150	5.07	10.66
180	5.21	10.80
210	5.07	11.36
240	4.93	11.36
270	5.14	11.22

Comparision of the ammonium concentration measured by CFS and HPLC.

Veen.	105 °C
Uiterw.	105 °C
Heide	105 °C
Veen.	40 °C
Uiterw.	40 °C
Heide	105 °C

Standard solution of 3 x 10^{-5} M NH_3

The Veen. 105 °C, Uiterw. 105 °C, Heide 105 °C, Veen. 40°C, Uiterw. 40 °C and Heide 105 °C soil samples were extracted with 0.01 M $CaCl_2$. The shaking time was 2 hours. After centrifuging, 20 ml of the clear supernatant was pipetted into 100 ml erlenmeyer flasks and measured by CFS. Another aliquot of the same soil extracts was pipetted and measured by HPLC. Since the measurement by HPLC took very long time (greater than 20 hours), all the soil extracts were measured again with CFS on the day after the extraction. The results are given in Table 4, in general, there is no difference between the NH^{+}_{4} concentration value obtained by both methods. The NH^{+}_{4} concentrations measured by CFS on the day of the extraction and one day after the extraction are equal, with exception for Veen soil dried at 40 °C. This is thought to be a wrong value.

Table 4. Comparision of NH^{+}_{4} concentration between CFS and HPLC.

Samples	NH^{+}_{4} conc. (mg N/kg soil)		
	CFS		HPLC
	first day	second day	
Veen. 105 °C	11.65	12.00	12.93
Uiterw. 105 °C	15.66	15.96	17.29
Heide. 40 °C	10.02	10.22	10.98
Veen. 40 °C	1.75	4.08	1.14
Uiterw. 40 °C	1.54	2.06	1.04
Heide. 40 °C	5.23	5.33	4.37
Stand. 3×10^{-5}	-	-	2.84×10^{-5}

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