

Ammonium analysis

Based on:

Weatherburn, M. W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Analytical Chemistry 39:971-974.

Sodium salicylate solution

6.8 g sodium salicylate
5 g sodium citrate
5 g sodium tartrate
0.025 g sodium nitroprusside
100 ml ultrapure water

Sodium hydroxide solution

6 g sodium hydroxide
100 ml ultrapure water

Bleach solution (make fresh each day)

0.2 ml bleach
9.8 ml sodium hydroxide solution

For resin bag extracts, the matrix is 0.1 M HCl/2.0 M NaCl. Extracts from unfertilized plots should follow the protocol for low concentrations and use the low standard curve. Extracts from fertilized plots should be diluted in matrix and run using the high protocol.

For low concentrations (0-5 ppm):

Add the following to each well:

80 μ l sample
60 μ l salicylate solution (add using multichannel pipet)
60 μ l bleach solution (add using multichannel pipet)

For high concentrations (1-10 ppm):

20 μ l sample
90 μ l salicylate solution (add using multichannel pipet)
90 μ l bleach solution (add using multichannel pipet)

Pipet up and down to mix well, incubate 50 min and read plate at 650 nm.

Standard curves: Dilute the 100 ppm stock solution to 10 ppm in a 1.5 ml centrifuge tube (150 μ l stock:1350 μ l matrix). Create the following standard curves in 1.5 ml centrifuge tubes.

High			Low		
Concen	μ l 10 ppm	μ l matrix	Concen	μ l 1 ppm	μ l matrix
0 ppm	0	1000	0 ppm	0	1000
0.5	50	950	0.05	50	950
1.0	100	900	0.10	100	900
2.0	200	800	0.20	200	800
5.0	500	500	0.50	500	500
10.0	1000	0	1.00	1000	0

Detection limit <0.05 ppm

Stock ammonium solution: 0.23585 g ammonium sulfate in 500 ml ultrapure DI water

Nitrate analysis

Based on:

Doane, T. A., and W. R. Horwath. 2003. Spectrophotometric determination of nitrate with a single reagent. *Analytical Letters* 36:2713-2722.

1 M HCl

Add 500 ml ultrapure water to 1 liter volumetric flask
Add 84 ml concentrated HCl to flask and swirl to mix
Bring to 1 liter with ultrapure water

Saturated vanadium chloride solution

Add 0.35 g vanadium (III) chloride to 50 ml of 1 M HCl; filter if necessary
CAUTION: the vanadium chloride powder is very reactive with air! Work quickly.

2% sulfanilamide solution

0.2 g sulfanilamide
10 ml of 1 M HCl

0.2% NED solution

0.02 g N-(1-naphthyl)-ethylenediamine dihydrochloride
10 ml ultrapure water

Reagent solution

50 ml saturated vanadium chloride solution
3.3 ml 2 % sulfanilamide solution
3.3 ml 0.2 % NED solution
400 ml DI water
Purge 17 ml aliquots with nitrogen or helium and store for up to 3 months frozen

For resin bag extracts, the matrix is 0.1 M HCl/2.0 M NaCl. Extracts from unfertilized plots should follow the protocol for low concentrations and use the low standard curve. Extracts from fertilized plots should be diluted in matrix and run using the high protocol.

For low samples, combine 100 μ l sample and 100 μ l reagent. For high samples combine 10 μ l sample and 160 μ l reagent. Tap microplate corner to mix well, incubate at least 5 h or overnight, and read plate at 540 nm.

Standard curves: Dilute the 100 ppm stock solution to 10 ppm in a 1.5 ml centrifuge tube (150 μ l stock:1350 μ l matrix). Create the following standard curves in 1.5 ml centrifuge tubes.

High			Low		
Concen	μ l 10 ppm	μ l matrix	Concen	μ l 1 ppm	μ l matrix
0 ppm	0	1000	0 ppm	0	1000
0.5	50	950	0.05	50	950
1.0	100	900	0.10	100	900
2.0	200	800	0.20	200	800
5.0	500	500	0.50	500	500
10.0	1000	0	1.00	1000	0

Detection limit <0.05 ppm

Stock nitrate solution: 0.3609 g potassium nitrate in 500 ml ultrapure water

Inorganic phosphorus analysis

Based on:

Lajtha, K., C. T. Driscoll, W. M. Jarrell, and E. T. Elliott. 1999. Soil phosphorus: characterization and total element analysis. Pages 115-142 in G. P. Robertson, D. C. Coleman, C. S. Bledsoe, and P. Sollins, editors. Standard Soil Methods for Long-Term Ecological Research. Oxford University Press, New York.

AMP solution

Add 250 ml ultrapure water to a 500 ml volumetric flask.

Add 53 ml concentrated sulfuric acid.

Dissolve 8.775 g ammonium para-molybdate and bring to volume.

Malachite green solution

Heat 400 ml ultrapure water to 80°C in an Erlenmeyer flask with stir bar.

Add 1.75 g polyvinyl alcohol and stir to dissolve.

Add 0.175 g malachite green and stir to dissolve.

Cool and bring to 500 ml volume in a volumetric flask.

Add 30 µl AMP solution to 150 µl sample and tap corner of microplate to mix. After 10 min, add 30 µl malachite green solution and pipet up and down mix. Do not place plate on shaker to mix- a nasty precipitate will form. Read microplate at 630 nm after 30 min.

Standard curves: Dilute the 50 ppm stock solution to 1 ppm in a 1.5 ml centrifuge tube (30 µl stock:1470 µl matrix). Create the following standard curve in 1.5 ml centrifuge tubes.

Concen	µl 1 ppm	µl matrix
0 ppm	0	1000
0.02	20	980
0.05	50	950
0.10	100	900
0.20	200	800
0.50	500	500

Detection limit <0.02 ppm

Stock phosphate solution: 0.2195 g oven-dried KH_2PO_4 in 1000 ml ultrapure water