Ammonium Protocol

•Ellen Esch, May 2019, adapted from Weatherburn, M. W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Analytical Chemistry 39:971-974. And http://allison.bio.uci.edu/protocols/.

•Detection limit is <0.05 ppm. If you need lower, update the standard curve.

•If you need to extract from soils, you can do that, usually 15 g soil, 100 ml 2M KCl shaken for 60 min, settled overnight, filtered via Hart, S.C., Stark, J.M., Davidson, E.A. & Firestone, M.K. (1994) Nitrogen mineralization, immobilization, and nitrification. Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties (eds R.W. Weaver, J.S. Angle & P.S. Bottomley), pp. 985–1018. Soil Science Society of America, Madison, WI, USA.

•NOTE: "matrix" is DI water for rivers/streams. For resin bags it is likely 0.1 M HCl/2.0 M NaCl, soil extracts KCl. (salinity matters)

Make solutions:

Sodium salicylate solution → 100 ml will do ~ 10 plates at [high], and ~15 plates at [low]

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

Sodium hydroxide solution → will make enough for ~10 days of running plates

- 6 g sodium hydroxide *cabinet
- 100 ml ultrapure water

Bleach solution (make fresh each day) → 100 ml will do ~ 10 plates at [high], and ~15 plates at [low]

- 0.2 ml bleach
- 9.8 ml sodium hydroxide solution

Stock ammonium solution (100 ppm) → make weekly

- Add 23.58 mg ammonium sulfate $(NH_4)_2SO_4$ to a 500 mL volumetric with nanopure
  - (0.01 g N / 1 L) * (132.14 g $(NH_4)_2SO_4$ / 28.01 g N) * (0.5 L) * (1000 mg / 1 g) = 23.58 mg $(NH_4)_2SO_4$
  - (desired ppm) * (percent N) * (desired volume) * (g to mg conversion) = mg $(NH_4)_2SO_4$ to add to 500 mL
  - 100 ppm N = 100 µg N / 1 mL = .01 g N / 1 L

Make standard curve:

1. Dilute the 100 ppm stock solution to either:
   •10 ppm; in a 1.5 ml centrifuge tube (150 µl stock:1350 µl matrix).
   •1 ppm, add 500 µl of 100 ppm stock solution to 50 ml volumetric flask and fill to the line with nanopure.
2. Create the following standard curves in 1.5 ml centrifuge tubes.

High				Low		
Std []	µl 10 ppm	µl matrix		Std []	µl 1 ppm	µl matrix
0 ppm	0	1000		0 ppm	0	1000
0.5 ppm	50	950		0.05 ppm	50	950
1.0 ppm	100	900		0.10 ppm	100	900
2.0 ppm	200	800		0.20 ppm	200	800
5.0 ppm	500	500		0.50 ppm	500	500
10.0 ppm	1000	0		1.00 ppm	1000	0

Run analysis:

NOTE: run samples in triplicate (or quadruplicate!!), and add the standard curve to one plate. Add the samples to the wells first, and then use the multichannel pipette to add the reagent. Make sure to label plates, and create a diagram for your sample layout. If samples turn yellow you have too high a concentration of ammonium (it is supposed to be blue/green); dilute sample and run again (ie 100 µl sample + 900 µl matrix)

For low concentrations (0-5 ppm), add the following to each well:

1. 80 µl sample
2. 60 µl salicylate solution (use multichannel pipet)
3. 60 µl bleach solution (use multichannel pipet)

For high concentrations (1-10 ppm):

1. 20 µl sample
2. 90 µl salicylate solution (use multichannel pipet)
3. 90 µl bleach solution (use multichannel pipet)

Tap corner of plate to mix well, cover with foil and incubate for 50 min. Read plate at 650 nm. (maybe 625 according to original paper)

Reporting results:

"Concentration of ammonium was determined colorimetrically using an Epoch microplate reader (BioTek, VT, USA) with the phenol-hypochlorite reaction method for ammonium (Weatherburn 1967). Samples were run in triplicate (or quadruplicate), and outliers were determined by xyz."