SOP: **Determination of Extractable Nitrate, Ammonium, and Phosphorus of Ion-Exchange Resin**

Overview:

The following standard operating procedure (SOP) for resin extraction is used to quantify the amount of nitrate, ammonium, and phosphorus from ion-exchange resin harvested from lysimeters in the field.

Nitrate quantification is accomplished via **Vanadium (III) reduction**. Vanadium (III) reduces nitrate into nitrite/nitric oxide when combined with a dilute acid solution[1,3]. The reaction of nitrate reduced by Vanadium (III) occurs as followed:

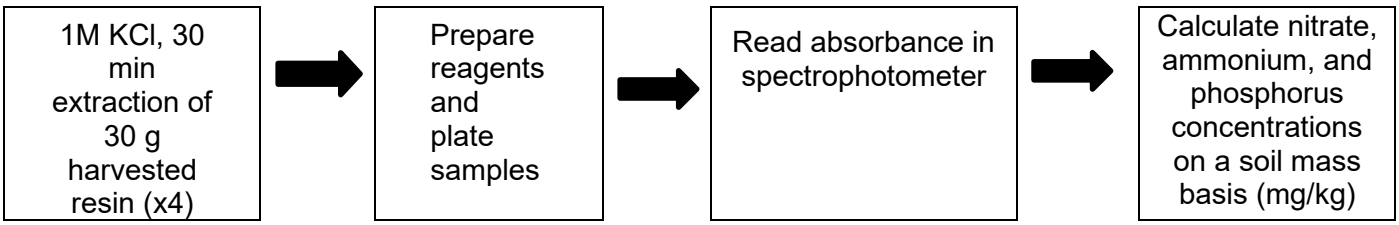
$$NO_3^- + 3V^{3+} + 2H_2O \rightarrow NO + 3VO^{2+} + 2H^+$$

By capturing both nitrite and nitric oxide with **Griess reagents** (sulfanilamide, N-(1-naphthyl)-ethylenediamine), a **pink dye** to each sample is produced, allowing for the colorimetric analysis of nitrate[1,2,4].

Ammonium quantification is accomplished via **the salicylate method.** Sodium salicylate is used as the **phenolic reagent** necessary for ammonium determination. Ammonium and salicylate react in the presence of a **nitroprusside catalyst**[1,2]. Citrate and sodium potassium tartrate are both added as **complexing agents** preventing the precipitation of metals. This is necessary for soil extracts but is not needed for water samples[1].

The hypochlorite addition results in the **chlorination** of the phenolic compound, leading to color development necessary for the colorimetric analysis of ammonium[2].

The Murphy and Riley colorimetric method was developed based on the observation that ammonium molybdate and potassium antimony tartrate react with diluted orthophosphate P solutions in an acid medium to form an antimony-phosphomolybdate complex (Pierzynsky, 2000). With the addition of ascorbic acid, the antimony-phosphomolybdate complex is placed in a reducing environment, the Mo is reduced and the complex turns blue. The blue color is measured by spectrophotometer at 882 nm wavelength. The light absorption is proportional to the orthophosphate P concentration in the sample.



Safety:

All standard safety protocols and online safety training via UIUC Division of Research Safety (DRS) are required.
Personal protection (PPE) for this procedure include:
Eye Protection: Safety goggles
Body Protection: Lab coat
Hand Protection: Gloves
Particularly hazardous substances:
Vanadium (II) chloride should be handled with care and under a fume hood, as this substance gives off corrosive fumes when exposed to moist air[1]. Once in solution, however, fumes will not occur. Any waste produced while handling Vanadium (III) chloride should be placed either in a labeled waste container or placed in a labeled solid waste bag according to DRS regulations.
Resin beads are corrosive and can cause serious eye damage, so handle with care and wear appropriate PPE.
Sodium Nitroprusside (nitroferricyanide) is toxic if swallowed. It should never come in contact with acidic solutions because hydrogen cyanide can be produced. Seal with parafilm and store in desiccator immediately after use. Make sure to check Safety Data Sheet if unsure about how to handle this chemical.
Concentrated hydrochloric acid should be handled in the fume hood. Specific details on these substances are incorporated in the **Detailed Procedure** below.

Instrumentation & Consumables:

Standards preparation

- 1 mL microcentrifuge tubes
- Nitrate standard
    - Location: Chemicals Only Fridge
- 1M Potassium Chloride (KCl)
- 1000 µL pipette and tips
- Ammonium standard
    - Location: Chemicals Only Fridge
- Phosphorus standard
    - Location: Chemicals Only Fridge

Sample preparation

- 250 mL Nalgene bottles
- 50 mL Falcon Tubes
- 10 mL pipette tips

Reagent preparation

- Analytical balance (two decimal places sensitivity)
- 1M Potassium Chloride (KCl)
- Nitrate
    - Solution 1
        - 125 mL Erlenmeyer flask wrapped with tin foil
        - Vanadium (III) Chloride
            - Location: Refrigerator, S-23
    - Solution 2
        - 500mL Erlenmeyer flask wrapped with tin foil
        - Sulfanilamide
            - Location: Organic reagents shelf
        - N-(1-naphthyl)ethylenediamine dihydrochloride (NED)
            - Location: Organic reagents shelf
- Ammonium
    - Reagent A
        - 500 mL Erlenmeyer flask wrapped with tin foil
        - Sodium Salicylate
            - Location: Organic reagents shelf
        - Sodium Citrate
            - Location: Organic reagents shelf
        - Sodium Tartrate
            - Location: Organic reagents shelf
        - Sodium Nitroprusside
            - Location: Reagent desiccator
    - Reagent B

        - 250 mL Nalgene bottle
        - Sodium Hydroxide
            - Location: Inorganic reagents shelf
        - Bleach (5.25% - 6.5% Sodium Hypochlorite)
            - Location: Chemical Only Fridge
        - Small beaker (100 mL)
- Phosphorus
    - Murphy-Riley Solution A
        - 1L volumetric flask
        - Ammonium molybdate
            - Location: Organic reagents shelf
        - Antimony Potassium Tartrate
            - Location: Small chemical desiccator
        - H_2SO_4
            - Acid cabinet under S27 fume hood
    - Murphy-Riley Solution B
        - Ascorbic Acid
            - Location: Organic Reagents Shelf

Colorimetry

- Costar 96 well microplates
- Microplate spectrophotometer
- Pipettes and tips (20 µL – 200 µL)

Detailed Procedure:

I. Sample Preparation

1. Transfer the resin beads from the bags to a 250 mL Nalgene bottle (two bags from the same lysimeter combined into a bottle) and store in 4°C (refrigerator).
    i. If there is only one bag of resins found, instead of 2, scale the extraction down to have the same ratios but smaller volume.

Note: how duration of cold storage influences the nutrients adsorbed onto the resin beads is not known, but the study to test this is underway.

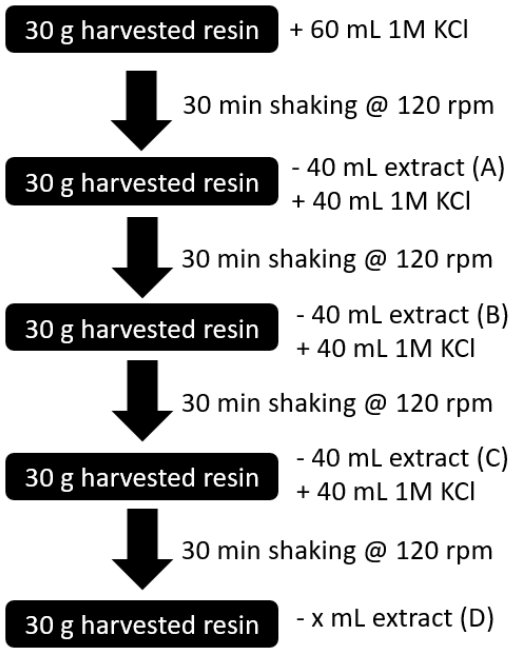
II. 1M KCl Preparation

1. In a 1L volumetric flask, dissolve 75g KCl into 1L 18.2 MΩ water.
2. Calculate in advance how much 1M KCl reagent you will need for all samples. Each sample will require 180 mL of 1M KCl.
    i. Using the proportion above in step 1, the amount of KCl needed for your desired volume can be calculated.
    ii. A slight excess of KCl should be made to ensure that you don't run low when trying to use the dispensette.
    iii. **Example:** For 40 samples 7.2 L of 1M KCl is needed. To make up 3.5L 1M KCl—

~262.5 g of KCl will be added into 3.5 L 18.2 MΩ water.

III. Sequential 1M KCl Extraction

1. To the transferred resin beads, add 60 mL of the 1M KCl extracting solution. Include blank samples, where no soil is added.
    i. It is recommended to run a maximum of 40 samples at a time.
    ii. If resin beads effervesce violently after 1M KCl addition, wait approximately 5 min before closing the lids.
2. Shake samples for 30 min on a reciprocating shaker, on low setting.
3. Remove from reciprocating shaker and transfer 40 mL of solution from each Nalgene bottle to a corresponding 50 mL Falcon tube (**Extract A).**
4. Add 40 mL 1M KCl to each Nalgene bottle from previous extraction and shake sample for 30 min on a reciprocating shaker, on low setting.
5. Remove from reciprocating shaker and transfer 40 mL of solution from each Nalgene bottle to a corresponding 50 mL Falcon tube (**Extract B).**
6. Add 40 mL 1M KCl to each Nalgene bottle and shake sample for 30 min on a reciprocating shaker, on low setting.
7. Remove from reciprocating shaker and transfer 40 mL of solution from each Nalgene bottle to a corresponding 50 mL Falcon tube (**Extract C).**



8. Add 40 mL 1M KCl to each Nalgene bottle and shake sample for 30 min on a reciprocating shaker, on low setting.
9. Remove from reciprocating shaker and transfer 40 mL of solution from each Nalgene bottle to a corresponding 50 mL Falcon tube (**Extract D).**
10. Centrifuge 50 mL Falcon tubes for 3 min at 4000 rpm.
11. In a new 50 mL Falcon tube, transfer 10 mL **Extract A,** 10 mL **Extract B,** 10 mL **Extract C,** and 15 mL of **Extract D**.
12. Centrifuged sample extracts should be stored in the refrigerator for 48 hours if being analyzed immediately, or frozen to preserve samples if immediate analysis is not possible.
13. NOTE: Instead of 40 mL from each extraction period to a separate tube, one could directly add 10 mL from extraction A, 10 mL from extraction B, 10 mL from extraction C, and 15 mL from extraction D directly into the same Falcon tube. For this method, still dispose of the remaining 30 mL from each extraction into a waste jug for a total of 40 mL removed each time.

IV. Standards Preparation

1. Follow the same standard preparation procedure for nitrate, ammonium, and phosphorus. **Nitrate will be used as an example.**
2. Pour a small amount of the nitrate standard into a 15 mL centrifuge tube or shallow container. This is to avoid any type of contamination on the reagent container.
3. Pipette the required amount of standard reagent (Table 1) to dilute in 1M KCl from 1000ppm to 100ppm into a 1 mL microcentrifuge tube. Proceed to pipette the required amount of 1M KCl and shake the mixture. Serial dilution then continues from the previous diluted standard.
4. Refer to Table 1 for examples of serial dilutions that can be used for creating a standard curve. The amounts will change depending on the required detection range.
5. Once the standards are ready, they should be treated as samples when it comes to plating them on the well microplate. Follow the exact colorimetry procedure for preparing samples for the standards. Make sure to remember their location on the plate, since the absorbance will be needed to create the curve to convert absorbance readings of the samples to concentrations.

Table 1. Standard dilution

NOTE: Serial dilution consists of performing the same dilution step repeatedly using the previous diluted solution as the input to the next dilution in each step. To create a serial dilution a concentration factor and a dilution factor are needed. These concepts are explained in the following formulas:

$$Concentration\ factor = \frac{volume_{initial}}{volume_{final}}$$

$$Dilution\ factor = \frac{1}{concentration\ factor}$$

The purpose of using serial dilutions is making the standards with known concentrations to create a standard curve, also known as calibration curve. This tool represents the relationship between two quantities. In this case, it assigns an estimated concentration to the absorbance value of the sample. Without the calibration curve, the absorbance values don't have a meaning. All the absorbance values of the samples must lay between the lowest and the highest absorbance values of the created standards. This means that the concentrations of the standards should be planned around the expected/estimated range of nitrate concentrations of the samples. However, a standard curve involving colorimetry is usually only linear until a certain concentration. In this case, samples outside the range of the standard curve need to be diluted with 1M KCl (e.g., 0.2 mL extract + 0.8 mL 1M KCl to yield 5x dilution) to bring the concentration down to the range of the standard curve, and the sample concentrations will need to be corrected for the corresponding dilution factor.

Standard concentration (ppm)	Amount of 1M KCl (µL)	Serial dilution of standards
		Amount of NO_3^- standard (µL)
100	900	100 of 1000ppm
20	800	200 of 100ppm
10	500	500 of 20ppm
5	500	500 of 10ppm
2.5	500	500 of 5ppm
1.25	500	500 of 2.5ppm
0	500	-

V. Colorimetry Reagents

1. Nitrate
    i. Solution 1 (**Make FIRST**, and wrap in aluminum foil)
        1. Pour 400 mL 18.2 MΩ water into a 500 mL Erlenmeyer flask. Add:
            a. 0.2 g sulfanilamide
            b. 0.01 g N-(1-naphthyl)-ethylenediamine
        2. Swirl until completely dissolved.
    ii. Solution 2 (**Make LAST**, and wrap in Aluminum foil)
        1. Make 1M HCl by diluting concentrated HCl with 18.2 MΩ water.
        2. Underneath the fume hood is storage for concentrated HCl.
            a. Transfer small amount of concentrated HCl from large container to a smaller container (e.g. 15 mL Falcon tube).
        3. Pour approximately 25 MΩ water into a 50 mL volumetric flask, then add **4 mL** of concentrated HCl. Dilute up to 50 mL in 18.2 MΩ water.
            a. **NOTE:** ADD WATER BEFORE ACID. AT LEAST 13 mL OF WATER IS NEEDED BEFORE ADDING ACID.
        4. Pour the 50 mL of 1 M HCl into 150 mL Erlenmeyer flask.
        5. Measure out 0.4 g (+- 0.2 g) of Vanadium (III) chloride (in fridge, S-23).
            a. NOTE: Always wear gloves, and always measure out in fume hood. A portable balance must be moved into fume hood for measurement. Use a thin weighing paper and a spatula. Any waste produced should be placed in labeled waste container or labeled solid waste bag according to DRS regulations.
        6. Add Vanadium (III) to HCl. Wrap 150 mL Erlenmeyer flask in aluminum foil. Swirl until completely dissolved.
    iii. Add Solution 1 to Solution 2

1. Store reagent in Nalgene bottle, covered with aluminum foil. Reagent can last up to 2-3 weeks in the refrigerator, or indefinitely in a freezer.

2. Ammonium
    i. Reagent A (Wrap in Aluminum foil)
        1. In a 500 mL Erlenmeyer flask, add:
            a. 100 mL water
            b. 6.5 g sodium salicylate
            c. 5 g sodium citrate
            d. 5 g sodium tartate
            e. 0.025 (+/- 0.005) g sodium nitroprusside (nitroferricyanide)
        2. NOTE: Always wear gloves when handling nitroprusside. Wrap in parafilm, and store in desiccator immediately after use
        3. Mix solution thoroughly, until completely dissolved. This may include the use of a magnetic stirrer.
        4. Solution 1 can be stored in a Nalgene bottle. Store Reagent A separately from Reagent B.
    ii. Reagent B
        1. In a 150 mL Erlenmeyer flask, add:
            a. 100 mL 18.2 MΩ water
            b. 6 g NaOH
            c. 2 mL Bleach (sodium hypochlorite)
        2. Percent sodium hypochlorite varies by bottle, but within the range of 5-6.5% can be utilized.
        3. Pour approximately ~3-5 mL bleach into a beaker, and pipette out 2 mL into Erlenmeyer flask.
        4. Store Reagent B in a Nalgene bottle. Shelf life for Reagent B is indefinite but is recommended to be replaced every 2-4 months. Store Reagent A separately from Reagent B.

3. Phosphorus
    i. Murphy-Riley Solution A
        1. Dissolve 4.3 g ammonium molybdate in 400 mL of deionized water in a 1-litre beaker.
        2. Dissolve 0.40 g antimony potassium tartrate in 400 mL deionized water, then add to the ammonium molybdate solution in the beaker.
        3. Slowly and carefully, with stirring, add 54 mL conc. H_2SO_4.
        4. Allow to cool and make to 1000 mL with deionized water. Mix well and store in a dark bottle in a refrigerator. *The reagent is stable for 4 weeks at 4°C
    ii. Murphy-Riley Solution B
        1. Ascorbic acid, 1%: Dissolve 1.00 g of ascorbic acid in 100 mL deionized water. Make a fresh solution daily as needed.

iii. Final Murphy-Riley (MR) reagent: combine 56 mL of solution B + 44 mL of solution A, and mix (should turn to light yellow color).
iv. 5% NaOH (for adjusting pH for colorimetry)

VI. Colorimetric Analysis

1. Nitrate
    i. Preparing samples with reagents (Table 2) into 96-well plates OR cuvettes.
        1. Add Sample
        2. Add Reagent
        3. Invert (if using cuvettes)
        4. Leave samples overnight (12-16 hours)
        5. Read at 540 nm
2. Ammonium
    i. Preparing samples with reagents (Table 3) into 96-well plates OR cuvettes.
        1. Add Reagent A
        2. Add Sample
        3. Add Reagent B
    ii. Invert (if using cuvettes)
    iii. Let samples sit (1-4 hours)
    iv. Read at 650 nm

3. Phosphorus
    i. Conduct colorimetry using a 3:7 ratio of extracts (and standards!) and Murphy-Riley reagent. The reaction generally takes >20 minutes.
    Note: different ratio of extract to Murphy-Riley reagent may be used, but make sure the ratio used is consistent across samples and standards.
        1. Example volumes that have worked in the past:
            a. Cuvette: 300 µL Mehlich III extract: 700 µL Murphy-Riley reagent
            b. 96-well plate: 60 µL Mehlich III extract: 140 µL Murphy-Riley reagent
        2. Read at 882 nm

Table 2. Nitrate Reagent and sample proportions

<1 ppm (LL 0.005 ppm)	**1-5 ppm (LL 0.05 ppm)**	**1-10 ppm (LL 0.07 ppm)**	**1-20 ppm (LL 0.07 ppm)**	**1-50 ppm**
Cuvettes------	**Cuvettes------**	**Cuvettes------**	**Cuvettes------**	**Cuvettes------**
500 uL reagent	1000 uL reagent	1000 uL reagent	1000 uL reagent	3500 uL reagent
500 uL sample	100 uL sample	45 uL sample	20 uL sample	30 uL sample
Microplates-----	**Microplates-----**	**Microplates-----**	**Microplates-----**	**Microplates-----**
150 uL reagent	273 uL reagent	287 uL reagent	294 uL reagent	297 uL reagent
150 uL sample	27 uL sample	13 uL sample	6 uL sample	3 uL sample
			*Most commonly used	

Table 3. Ammonium reagent and sample proportions

<1 mg/L (LL 0.02 mg/L)	**<1 mg/L (High Ca/Mg)**	**1-5 mg/L (LL 0.2 mg/L)**	**1-10 mg/L (LL 0.2 mg/L)**	**1-20 mg/L (LL 0.5 mg/L)**
Cuvettes------	**Cuvettes------**	**Cuvettes------**	**Cuvettes------**	**Cuvettes------**
150 uL reagent A	200 uL reagent A*	200 uL reagent A	500 uL reagent A	500 uL reagent A
600 uL sample	800 uL sample	800 uL sample	80 uL sample	40 uL sample
150 uL reagent B	200 uL reagent B	200 uL reagent B	500 uL reagent B	500 uL reagent B
Microplates-----	**Microplates-----**	**Microplates-----**	**Microplates-----**	**Microplates-----**
50 uL reagent A	128 uL reagent A*	128 uL reagent A	139 uL reagent A	144 uL reagent A
200 uL sample	45 uL sample	45 uL sample	22 uL sample	12 uL sample
50 uL reagent B	128 uL reagent B	128 uL reagent B	139 uL reagent B	144 uL reagent B
				*Most commonly used

I. Clean up

1. Experiment
    i. Dial back the pipettes to their corresponding volume.
    ii. ***DISPENSETTE: CLEAN IMMEDIATLEY AFTER USE***
    iii. Make sure all reagents are back in the shelves.
    iv. Liquid waste should be kept in a labeled liquid waste container. Any solid waste from handling of Vanadium (III) chloride should be kept in a separate labeled waste bag.
    v. Liquid waste from nitrate reagents should be kept in a separate waste container. **DO NOT** combine or mix with ammonium waste.
    vi. Liquid waste from Ammonium reagents should be kept in a separate waste container. **DO NOT** combine or mix with Nitrate waste. Sodium Nitroprusside (nitroferricyanide) should not be combined with acidic solutions (see "Safety" section for more details).
    vii. Liquid waste from phosphorus colorimetry should be kept in a separate waste container.
2. Resin Bags
    i. If planning to reuse resin bags, they will need to be washed to remove resin beads and soil debris.
    ii. To wash the bags, have 4 open basin containers ready. Fill the first two with DI water, the third with nanopure water, and leave the fourth empty. Place the dirty resin bags into basin 1 to soak (~15 minutes). Transfer some to basin 2, fold resin bags inside out and begin to massage the bags to clean the debris out (similar to if you were handwashing laundry). Once clean (there will still be some staining remaining), transfer to basin 3 and massage again in nanopure water. Once done, move to basin 4 to air dry.
3. Nalgene Bottles
    i. To clean the Nalgene bottles used in the extraction, first dump the remaining KCl and resin beads into a container (will need to be picked up by DRS). Then wash the Nalgene bottles thoroughly with soap and water (the beads can stick to the bottles so make sure none are remaining). Once washed, put them through an HCl acid bath rinse.

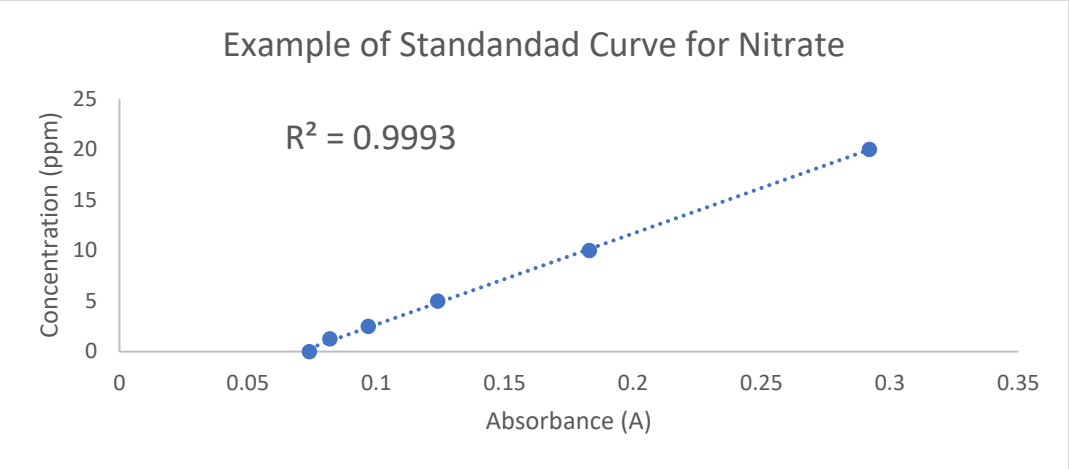
II. Calculations

Measurement of nitrate is usually expressed in units of mg/L. To calculate. See corresponding Excel calculation template.

1. Generate a scatter plot graph for the standard curve. Add a trend line and check if the R^2 value is acceptable (R^2 >0.99). An example of the absorbance values and graph is provided below.

Table 3. Example of recorded absorbance values from standard samples analyzed through the spectrophotometer.

Absorbance values	0.074	0.082	0.097	0.124	0.183	0.292
Concentration of standards (ppm)	0	1.25	2.5	5	10	20



2. **Note** which original standard solution you used. Nitrate standard can either be in mg NO_3-N/L or mg NO_3/L, and concentration differs depending on the unit of expression. For example, 1000 mg NO_3/L is approximately 226 mg NO_3-N/L. The same principle applies to ammonium standards (NH_4 and NH_4-N are different units).

Example 1. Sample conversion from absorbance to mg/L = ppm

	A	B	C	D
1	Absorbance (A)	Concentration (ppm)	Samples (A)	Samples (ppm)
2	0.074	0	1.329923274	113.5300274
3	0.082	1.25	1.150895141	97.39359339
4	0.097	2.5	1.355498721	115.8352322
5	0.124	5	1.636828645	141.1924856
6	0.183	10	1.150895141	97.39359339
7	0.292	20	1.023017903	85.86756912
8				
9	**Green (left two) columns are from standard curve. Orange (right two)**			
10	**columns are from sample readings. The standard curve is used to convert**			
11	**the sample absorbance (mg/L) into concentration (ppm).**			
12				
13	=TREND(known_ys, known_xs,new_xs)			
14	=TREND(concentration_values,absorbance_values,sample_value)			
15	=TREND(B$2:B$7,A$2:A$7,C2)			
16	LOCK the "concentration_values" and "absorbance_values"			
17	i.e., add the dollar sign after each letter. DO NOT lock sample value.			
18				

3. After converting the absorbance values to the corresponding concentrations (mg/L), as described above, the obtained concentration values should be multiplied by total volume of extraction (0.18 L) to yield cumulative amount of nitrate extracted (in either NO_3-N or NO_3 depending on the standard used). Also, if the extract was diluted as described above, multiply the concentrations by the corresponding dilution factors.

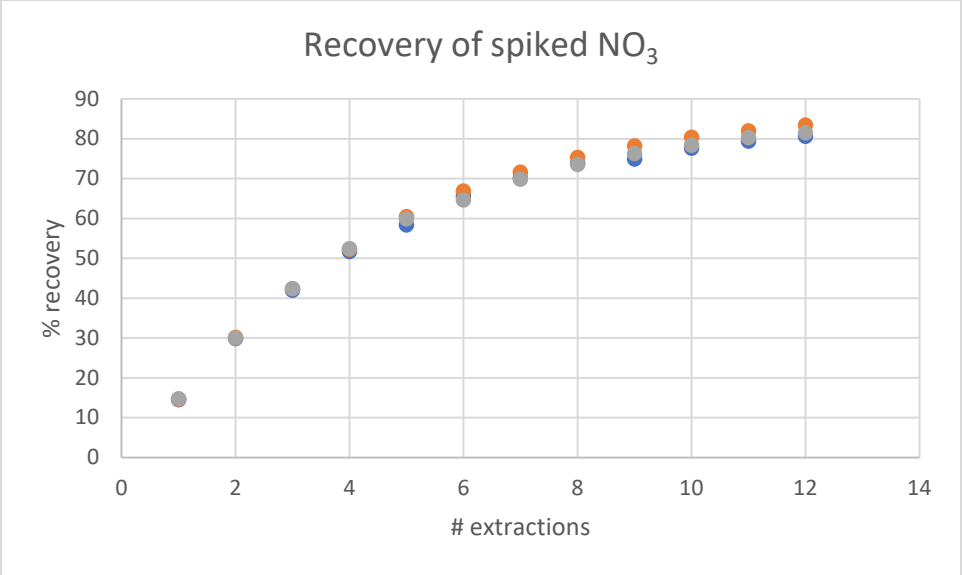
   In case you are curious, the sequential extraction procedure described in this SOP results in 'carryover' of extract from one step to another, given that not all of the added 60 mL 1M KCl is removed at each extraction step. This carryover can be accounted for by the formula below:
   C1, C2, C3, and C4 correspond to the concentration of the extract from 1st, 2nd, 3rd, and 4th extraction steps, respectively.

$$cumul\ NO3 = C1 \times 0.06L + (C2 \times 0.06 - C1 \times 0.02) + (C3 \times 0.06 - C2 \times 0.04) + (C4 \times 0.06 - C3 \times 0.04)$$

$$cumul.NO3\ (mg) = 0.04(C1 + C2 + C3 + 1.5C4)$$

$$cumul.NO3\ (mg) = \underbrace{0.04 \times 4.5}_{\text{Total extraction volume (60 + 40 + 40 + 40) mL = 0.18 L}} \times \underbrace{\left(\frac{C1 + C2 + C3 + 1.5C4}{4.5}\right)}_{\text{Concentration of mixture of 4 extracts (1:1:1:1.5)}}$$

4. **Note** that the values obtained in the previous step are cumulative amount of nitrate **extracted**, not the total amount of nitrate that was adsorbed to the resin, which is what we are interested in to estimate total amount of nitrate leaching.
5. To correct for the incomplete recovery, a separate experiment involving the spiking of fresh exchangeable resin bags (with the same resin beads used for the lysimeters) with known amount of nitrate (ideally similar to the amount expected in your resin samples) and subsequently extracting with the exact same way as described above should be conducted.
   i. The described extraction method is shown to recover at least 50% of nitrate adsorbed on the resin so far, but the recovery can depend on the concentration adsorbed on the resin. Thus, the recovery characterization specific to each resin extraction method (e.g., extraction ratio, number of extractions, extracting solution, and resin beads used) is critical for accurately estimating leaching. Figure below shows data from a spike-recovery experiment testing % recovery of spiked nitrate with up to 12 consecutive extractions. In this example recovery data, you can see that at least 50% recovery is achieved after four consecutive extractions.



6. The value obtained above can then be converted to the unit of kg NO_3-N /ha. To perform this calculation, the diameter of PVC coupling used for resin lysimeter is needed. Below is an example calculation to obtain a conversion factor, assuming 50% recovery of nitrate by extraction from the harvested resin lysimeter (in this example, concentration of the original standard was expressed as mg NO_3/L, not NO_3-N/L, so 14/62 was added to account for this):

$$\frac{mgNO_3\ per\ lysimeter\ to\ kg\ N\ per\ hectare}{\frac{cum\ extracted\ mgNO_3}{(3cm)^2\pi} \times \frac{1kg}{10^6 mg} \times \frac{10^8 cm^2}{1\ ha} \times \frac{14\ kg\ N}{62\ kg\ NO_3}}$$

$$= \mathbf{0.7986}\ (conversion\ factor)$$

$$Assuming\ 50\%\ recovery, \mathbf{1.597}$$

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