

Introduction

Over the last two decades, low levels of pharmaceuticals and personal care products have been detected in natural waterways and soil systems. Although concern of overt toxicity is low, pharmaceuticals are designed to elicit highly specific physiological responses, and uncontrolled release into the environment may produce unexpected biological responses in target and non-target organisms. Antibiotics, in particular, are a growing area of concern due to selection and development of drug-resistant bacteria, which are a significant public health threat, especially with bacteria that are resistant to multiple antibiotics like methicillin-resistant *Staphylococcus aureus*. Consequently, quantification of antibiotics in soil and water systems is necessary to completely understand the impacts of these compounds in the environment.

The main pathway human antibiotics enter the environment is through release of wastewater treatment plant (WWTP) effluent, either directly into waterways or spray irrigated over agricultural lands. Penn State has a unique wastewater-renovation cycle site, known as the Living Filter, where WWTP effluent is spray irrigated on agricultural, grass and forestlands year-round. This site has been in full operation since the 1980's and offers an optimal location for studying antibiotics within environmental compartments.

Objective

To develop optimal extraction and clean up procedures for water and soil samples that allow accurate and simultaneous quantification of four antibiotic residues of interest: sulfamethoxazole (SXM), trimethoprim (TRM), ofloxacin (OFL), and lincomycin (LIN) at Penn State's Living Filter.



Spray field at Penn State's Living Filter

Methods: Water Samples

- Water samples were collected at the Living Filter and University Park WWTP to analyze for the four antibiotics of interest.
- Triplicates of samples were collected from influent, effluent, and six wells near the Living Filter and quantified (Table 1).
- SPE recoveries for spiked water samples were 100% for SXM, 75% for TRM, 32% for OFL, and 82% for LIN.
- Sample quantification was performed using a Waters Xevo TQS, UltraPerformance LC® (UPLC) tandem mass spectrometer (UPLC-MS/MS) at the Metabolomics Facility at Penn State.

Table 1. Antibiotic concentrations in water samples

Antibiotics	Water Samples (ng/L)			
	Influent	Effluent	Groundwater ‡	
			Well 1	Well 2
SXM	711.6 ± 66.4	562.8 ± 61.8	84.7 ± 10.9	70.3 ± 4.5
TRM	488.2 ± 166.4	22.1 ± 9.2	ND	ND
OFL	35.7 ± 10.0	44.9 ± 1.2	ND	ND
LIN	39.6 *	ND	ND	ND

ND - Not Detectable

‡ - Four additional wells were samples, but antibiotic levels not detected.

* - Only two samples.

Methods: Soil Samples

Study Design

- Four antibiotics (SXM, TRM, OFL, and LIN) were extracted from spiked soils using both batch and Accelerated Solvent Extraction (ASE) procedures with four different solvent mixtures.
- Antibiotics were selected based on presence in effluent at the Living Filter, frequency found within WWTP effluent and natural waterways nation-wide, and risk of increased antibiotic resistance.
- Solvent mixtures were selected based on previous research, as well as the chemical characteristics of the antibiotics.

Lab Analysis

Soil Equilibration: Solutions of 18 Mohm water with CaCl₂ (0.005 M) and sodium azide (250 mg/L) were spiked with 1 µM of each antibiotic (SXM: 253.3 µg/L, TRM: 290.3 µg/L, OFL: 361.4 µg/L, LIN: 406.5 µg/L). Solutions (100 mL) were added to 50 g of A horizon of Hagerstown silt loam soil and equilibrated for 24 hours. Equilibration solution was decanted after centrifugation. Concentrations of antibiotics in 100 mL of recovered equilibrated solution were SXM: 13.25 µg, TRM: 1.06 µg, OFL: 0.076 µg, and LIN: 18.30 µg. Assumed concentrations adsorbed to the soil were SXM: 12.08 µg, TRM: 27.97 µg, OFL: 36.1 µg, and LIN: 22.3 µg.

Soil Extractions:

- Batch Procedure:** Antibiotics were extracted from spiked soils by the addition of 100 mL of extraction solvent followed by equilibration for 1 hour and removal of extraction solvent after centrifugation. Extraction procedure was, then, repeated for a total of two extractions.
- ASE Procedure:** Each cell was filled sequentially with 2 g of sand, 12 g of soil and 2 g of sand. ASE extraction settings were 100 °C, 1500 psi, three 10 minute static cycles, 60% flush volume and 60 s purge. One extraction volume was obtained (varied from 10-20 mL).
- Solvents:** (1) Water, pH 2.8, (2) 50/50 ACN/H₂O, pH 2.8, (3) Methanol, pH 2.5, (4) 20/80 Methanol/H₂O, pH 2.7

SPE: For batch procedures, a subset (30 mL) of each recovered extraction solvent was cleaned up by SPE, while the total extraction volume from the ASE procedure was run through the SPE procedure. After clean up and evaporation, samples were brought up in 1 mL of mobile phase with an internal standard.

Quantification: Waters Xevo TQS, UPLC-MS/MS at Penn State's Metabolomics Facility (shown to the right).



Summary

- Summary of soil extraction results can be seen in Table 2.
- Overall, EPA 1694 methodology is not suitable for recovery of OFL.
- For the extraction of SXM, TRM, and LIN, a single solvent and extraction procedure does not optimally extract them simultaneously.
- ASE procedure consistently provided higher recoveries as compared to batch procedure, except when extracting with pH adjusted water (Fig. 1).

Table 2. Summary of results for extracting antibiotics from soils

Antibiotic	Solvent	Method	Extraction Recovery [µg]*
SXM	50/50 ACN/H ₂ O	ASE	13.65 (103.6%)
TRM	50/50 ACN/H ₂ O	ASE	10.94 (39.1%)
OFL	50/50 ACN/H ₂ O	ASE	0.24 (0.67%)
LIN	20/80 MeOH/H ₂ O	ASE	12.10 (54.3%)

* Antibiotics were extracted from 50 g of soil. Solvents are pH adjusted.

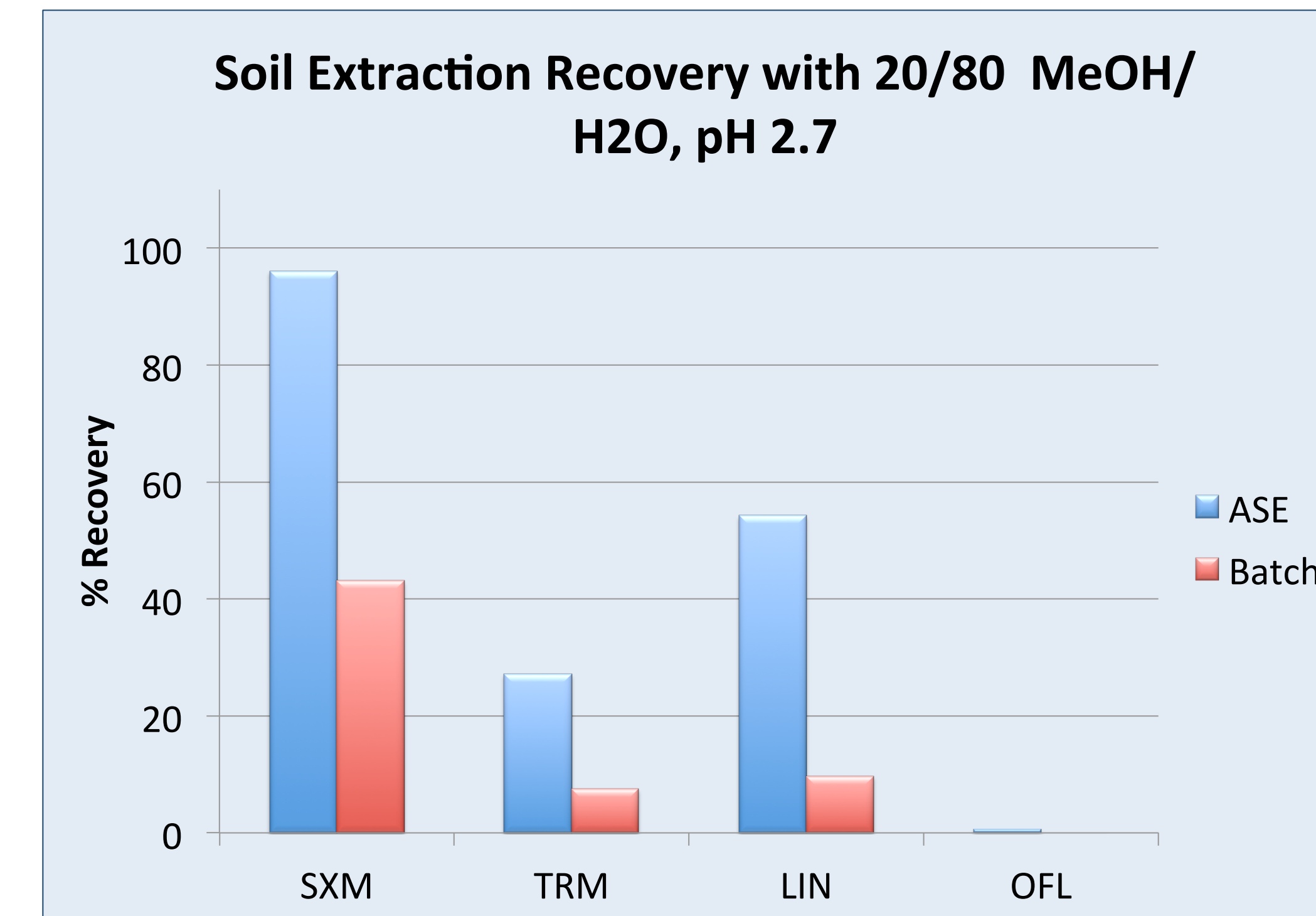
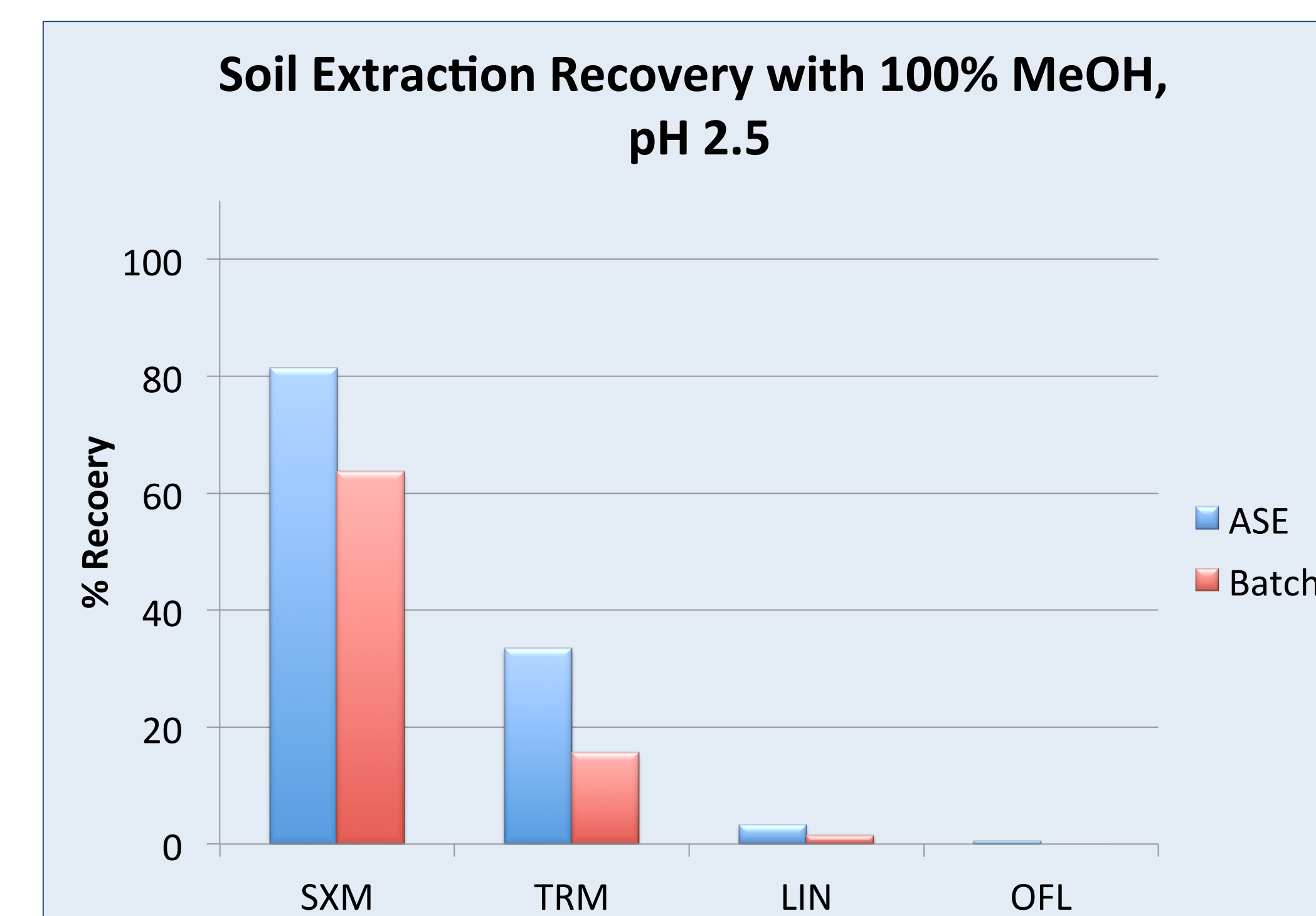
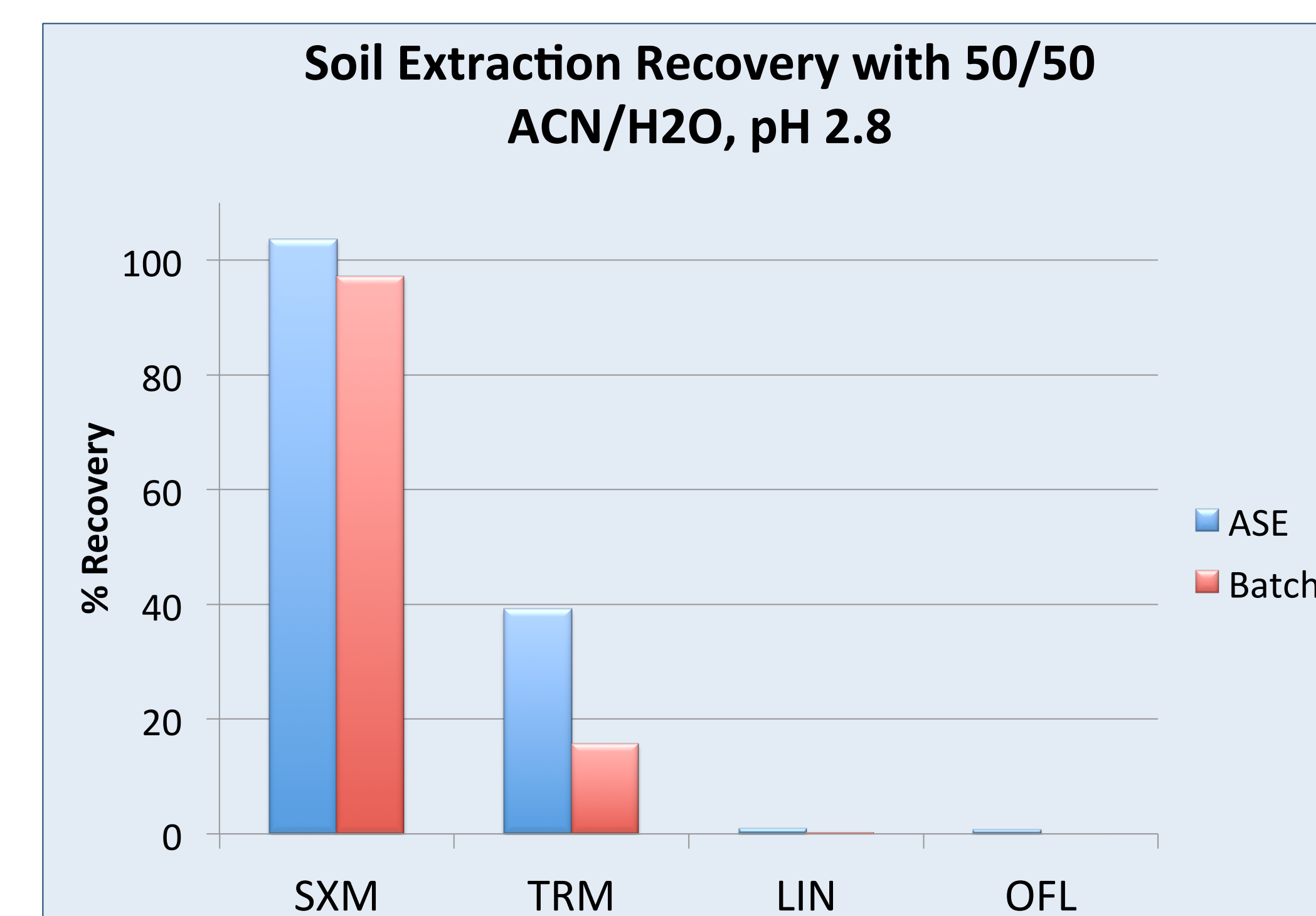
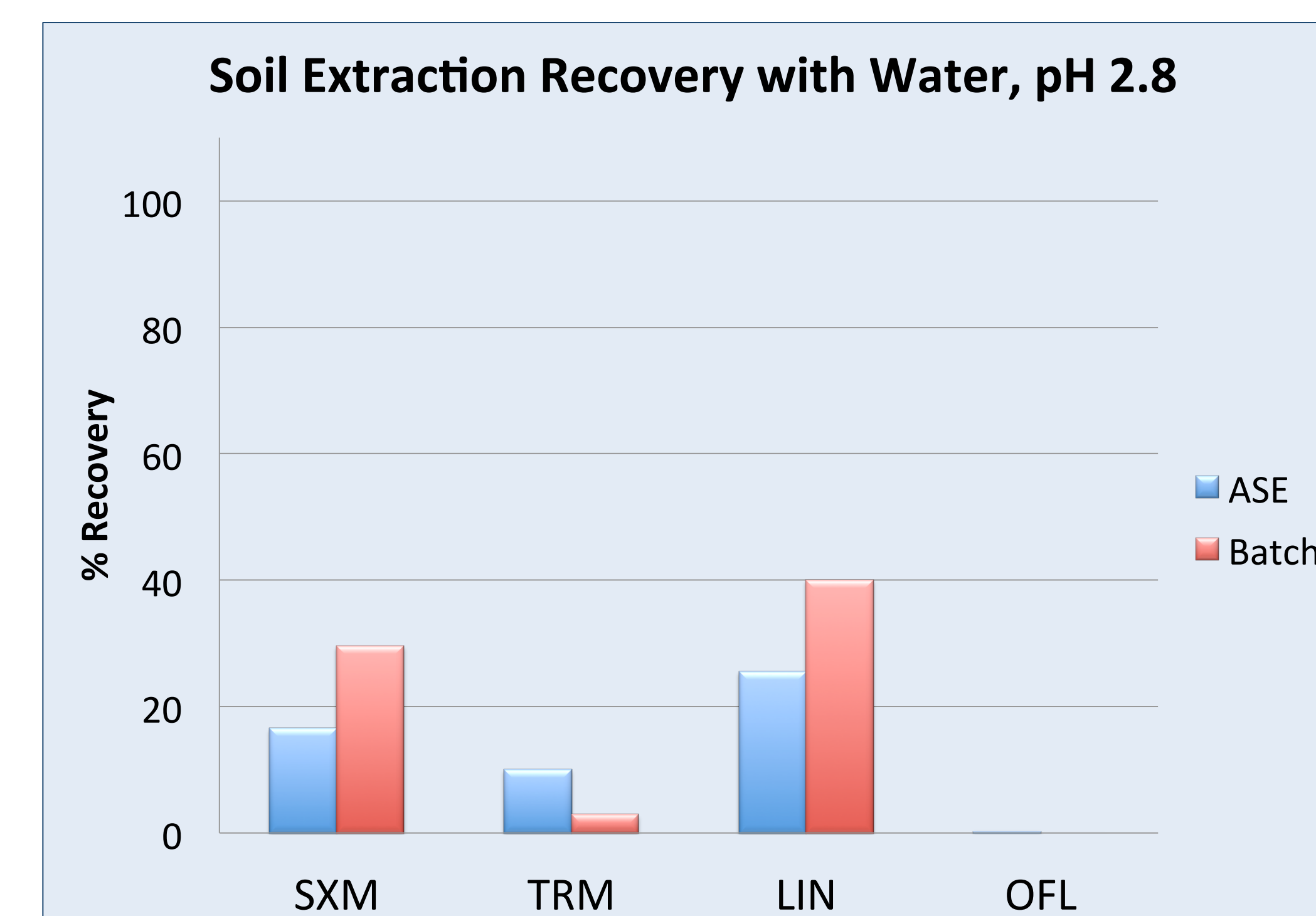


Figure 1. Comparison of Batch and ASE soil extraction methods using different extraction solvents for four antibiotics: sulfamethoxazole (SXM), trimethoprim (TRM), ofloxacin (OFL), and lincomycin (LIN).

Acknowledgments

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