Extraction of phenolic compounds from soils

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Key Points

1. Total phenolics extracted from soil with acidified methanol can be used to identify differences among treatments.
2. While little difference was observed for plant material, MSA-MeOH extracted more TP than 70% aqueous acetone from soil.
3. Further research is required to develop a means for distinguishing between phenolics from recent plant inputs and those that are the products of decomposition or an artifact of other oxidizable compounds in soil.

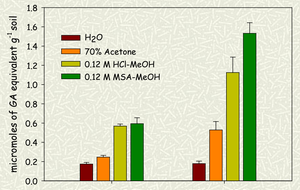
Problem: Understanding the composition and amount of phenolics in soil is important for studies of soil organic matter formation and nutrient cycling. Colorimetric methods such as **Modified Prussian Blue**[1] (PB) or the **Folin-Ciocalteu**[2] (FC) are commonly used to measure total phenolics (TP) but some phenolic compounds, including tannins, can sorb or complex with the soil making them difficult to extract.

Methodology: Air-dried soils were extracted with water (H_2O), 70% aqueous acetone (acetone), or methanol (MeOH) acidified with either 0.12M Hydrochloric (HCl) or 0.12M Methanesulfonic Acid (MSA) under standardized assay conditions (1:10 w/v 1 hour shake at 23 ^{o}C). After centrifugation, supernatants were assayed by modified Prussian Blue analysis, expressed as gallic acid (GA) equivalents .

Modified Prussian Blue Assay (PB): Based on the reduction of Fe(III) to Fe (II) by oxidizable compounds (presumably phenolics) in the presence of ferricyanide, the PB assay produces a colorimetric response (measured at A700). We prefer the PB method over the FC method for quantification of TP in soils due to interfering precipitates that can occur with FC reagents and acidified methanol extracts. Also, the PB assay is faster. However, both assays can be compromised by the presence of other non-phenolic oxidizable compounds in soil extracts .

Extraction of TP from soil varies with solvent

Only small amounts of PB phenolics were extracted from soil by water (below) while acidified methanol extracted more TP than acetone. While greater quantities of TP were extracted from woodland soil, the difference between the MSA-MeOH and HCl-MeOH extractants was less pronounced in pasture soils.

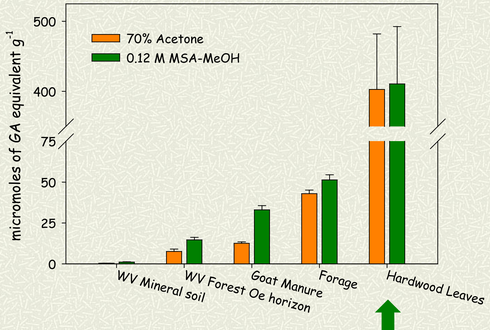


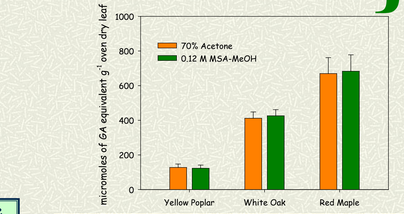
Total phenolics in forages and resulting manure were 1-2 orders of magnitude greater than mineral soil.

The amount of TP varies among sources

The magnitude of TP in mineral soil was compared to other sources. Phenolics were determined in forest Oe horizons, manure, forages, and overstory hardwood leaves after extraction with acetone or MSA-MeOH under standard assay conditions (1:20 w/v, 30 minutes ultrasonic H_2O bath at 23 ^{o}C).

Comparison among Extractants



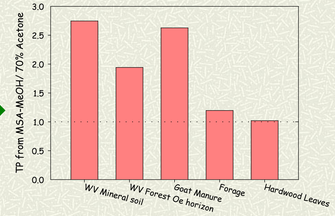


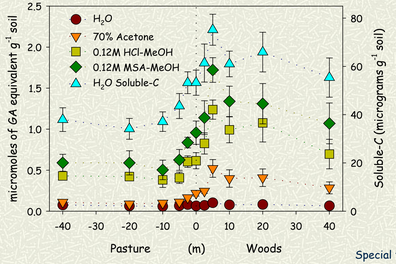
Total phenolics in hardwood leaves varied by species and were 2-3 orders of magnitude higher than mineral soil.

Efficacy of solvents varies among sources

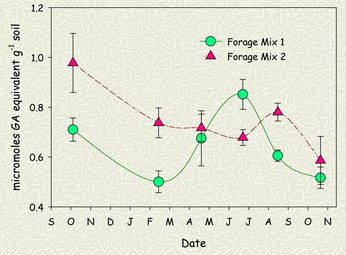
While little difference was observed for plant material, MSA-MeOH extracted 2-3 times more TP from mineral soil and goat manure than acetone.

These patterns may indicate variations in the composition or distribution of phenolics within the different sources, or that the extraction method needs further refinement.

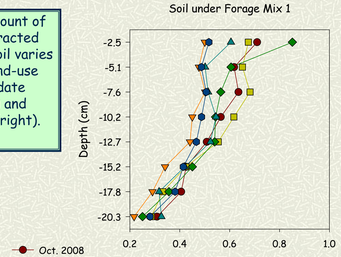


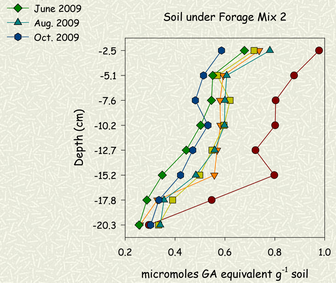


Total Prussian Blue Phenolics in soil (2.5 cm)



The amount of TP extracted from soil varies with land-use (left), date (above) and depth (right).

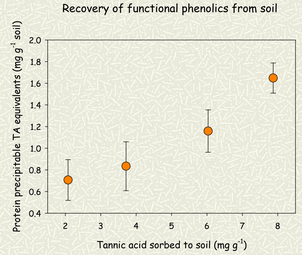




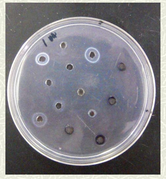
Some extracted tannins appear to retain functionality

Pasture soils (n=5) treated with increasing amounts of Tannic Acid (TA) were washed (2X), air-dried and extracted with MSA-MeOH (1:2 w/v). Treatment solutions and washes were assayed for TP and soluble carbon to estimate the amount of TA sorbed onto soils.

Recovery of functional phenolics from soil



Soil extracts were able to precipitate protein in an agar medium (radial diffusion assay[3], below), indicating the recovery of functional tannins.



1) Graham, H. D. 1992. *J. of Ag. & Food Chem. 40*(5), 801-805.
2) Swain, T. & Hillis, W.E. 1959. *J. Sci. Food Agric.* 10 (1) 63-68.
3) Hagerman, A. E. 1987. *J. of Chem. Ecol.* 13(3), 437-449.

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