Folin Ciocalteu phenol protocol

Total water-soluble polyphenols – total phenols expressed as gallic acid equivalents GAE 1:20 compost: water (w:v) extract, filtered over 0.45 μ m.

MilliQ Extract > pH & EC, TOC, TdN, phenols

Prepare 40 ml extract at 1:20 (w:v) on dry wt equivalent (is approx.. 2.1 gr air-dry) Weigh sample in 50 ml tube, add 40,0 ml MilliQ water with dispenser, shake manually, shake 120 min at 50 rpm with end-over-end shaker (wheel of fortune). Let it rest overnight, next morning shake 30 min. Centrifuge 30 min at 2000 rpm.

pH & EC > 6.0 ml in small glass tube, measure EC first. Calibrate pH meter before use. Filter rest of the extract over 0.45 μm with filter box. Store in fridge.

Calibration curve (Waterhouse 2003)

Dissolve 0.50000 g gallic acid in 10 ml ethanol and then dilute to 100 ml with water (5 g/liter final). Dilute 1, 2, 5, and 10 ml to 100 ml with water to create standards with 50, 100, 250, and 500 mg/liter concentrations, respectively. Then prepare similar to sample. Linear equation, which can be used to assess specific absorptivity of sample.

Last one is too high! Maybe do 7 ml next time

Y=aX+b, Y is concentration, X is absorbance

Measurement (Blainski et al., 2013)

1,0 ml sample solution (diluted) + 500 μ L 2 N Folin-Ciocalteu reagent + 5,0 ml milliQ water + 6,0 ml anhydrous sodium carbonate 10.75% w/v > mix.

Sample dilution 1:5, when very high phenol content dilute 1:10 with milliQ water

Measure absorbance after 30 min at 760 nm, quartz or plastic cuvette

Calculation

Fill in absorbance in calibration eq., correct for dilution > gives concentration (mg/L= μ g/ml). Calculate to mg EGA/ kg dry weight equivalent, μ g/g=mg/kg

 $TPC = C * \frac{V}{m}$ TPC= total phenolic content, C=concentration, V= volume, m=mass