Video Article

Physical, Chemical and Biological Characterization of Six Biochars Produced for the Remediation of Contaminated Sites

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Abstract

The physical and chemical properties of biochar vary based on feedstock sources and production conditions, making it possible to engineer biochars with specific functions (*e.g.* carbon sequestration, soil quality improvements, or contaminant sorption). In 2013, the International Biochar Initiative (IBI) made publically available their Standardized Product Definition and Product Testing Guidelines (Version 1.1) which set standards for physical and chemical characteristics for biochar. Six biochars made from three different feedstocks and at two temperatures were analyzed for characteristics related to their use as a soil amendment. The protocol describes analyses of the feedstocks and biochars and includes: cation exchange capacity (CEC), specific surface area (SSA), organic carbon (OC) and moisture percentage, pH, particle size distribution, and proximate and ultimate analysis. Also described in the protocol are the analyses of the feedstocks and biochars for contaminants including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), metals and mercury as well as nutrients (phosphorous, nitrite and nitrate and ammonium as nitrogen). The protocol also includes the biological testing procedures, earthworm avoidance and germination assays. Based on the quality assurance / quality control (QA/QC) results of blanks, duplicates, standards and reference materials, all methods were determined adequate for use with biochar and feedstock materials. All biochars and feedstocks were well within the criterion set by the IBI and there were little differences among biochars, except in the case of the biochar produced from construction waste materials. This biochar (referred to as Old biochar) was determined to have elevated levels of arsenic, chromium, copper, and lead, and failed the earthworm avoidance and germination assays. Based on these results, Old biochar would not be appropriate for use as a soil amendment for carbon sequestration, substrate quality improvements or remediation.

Video Link

The video component of this article can be found at https://www.jove.com/video/52183/

Introduction

Biochar is a carbon-rich by-product produced during the pyrolysis of organic matter [1]. Interest, both publicly and academically, in adding biochar to soils, stems from its ability to improve soil quality and plant growth [2, 3], sustainably sequester carbon [4], and sorb harmful contaminants [2, 3, 5-7] whilst simultaneously offering alternatives for waste management and energy production by pyrolysis.

Biochars are being produced by numerous companies and organizations worldwide via different pyrolysis systems. Materials used for biochar production include (but are not limited to) woodchips, animal manure and construction wastes[1]. These differences are expected to alter the biochars' physical and chemical properties and thus their ability to improve substrates, promote long-term stability and increase sorption capabilities. Additionally, during the pyrolysis process the biochar may become unintentionally contaminated with metals, PAHs and PCBs as a result of contaminated feedstocks or inappropriate pyrolysis conditions. Therefore, before biochar can be applied on a large scale to the environment as a soil amendment, careful characterization of the biochar for contaminants, specific surface area, cation exchange capacity, earthworm avoidance and germination and others suggested by the International Biochar Initiative (IBI) must be conducted. In 2013, the first Standardized Product Definition and Product Testing Guidelines for Biochar, which sets standards for biochar physical and chemical characteristics, was published and made publically available.

Research has shown that biochar produced at a commercial greenhouse in Odessa, ON, Canada has the ability to significantly improve plant growth in intensely degraded soils and sorb persistent organic pollutants (POPs) such as PCBs [2, 3]. This biochar has been produced from three different feedstocks (*i.e.* organic matter sources) via a boiler system where the heat generated is used to warm their greenhouse operation during winter months.

This study provides characterization data pertinent to the production of biochar in a biomass boiler, and the use of biochar as a soil amendment. The objective of this study is to thoroughly characterize the physical, chemical and biological characteristics of six biochars according to

standards set by the IBI in their Standardized Product Definition and Product Testing Guidelines (Version 1.1) (2013). These characteristics will be linked, where possible, to the performance of each biochar as agricultural amendments and their ability to sorb contaminants.

Protocol

NOTE: Chemical analyses were conducted at the Analytical Services Unit (ASU) in the School of Environmental Studies at Queen's University (Kingston, ON). The ASU is accredited by the Canadian Association for Laboratory Accreditation (CALA) for specific tests listed in the scope of accreditation. Other analyses, including greenhouse trials, were conducted at The Royal Military College of Canada (Kingston, ON) in the Department of Chemistry and Chemical Engineering.

1. General Considerations

1. To ensure quality assurance and quality control, analyze an analytical blank and an analytical duplicate, a sample duplicate and a standard reference material with each batch of samples (maximum batch size 10) for the methods in the protocol.
2. Establish duplicate samples when sub-sampling from the original sample and go through the same preparation as the unknown samples. Ensure that duplicate values are within 20% of each other or repeat the analysis. Ensure that analysis outcomes of the blanks are below detection limits for the corresponding method. Standard reference material limits depended on the individual method but ensure that they are generally within 15–30% of the expected value.
   NOTE: In many of the methods described in the protocol, details are included on the suggested order of sample analysis including calibrants, blanks, high and low standards, and unknown samples. This is to ensure no cross contamination between samples and ensure a high standard to QA/QC.
   NOTE: Six biochars were produced at a commercial greenhouse and analyzed for chemical, physical and biological parameters. The names of each biochar reflect their production parameters or feedstock source (**Table 1**).

2. Test Category A: Basic Biochar Utility Properties

1. Moisture and Organic Matter Content
   1. Use the loss on ignition procedure outlined by Nelson and Sommers (1996).
      1. Include a sample duplicate and standard reference material (Ottawa Sand) for every 10 unknown samples.
      2. Label 50-ml beakers with heat resistant marker, oven dry them at 105 °C, allow them to cool then record weight.
      3. Weigh 2 g of air-dried sample into the oven-dried beaker. Dry sample at 105 °C for 24 hr, then remove from the oven and allow to cool.
      4. Once cool, weigh the beaker and the sample (X = weight of dried sample - weight of beaker).
      5. Place the sample in the muffle furnace and heat for 16 hr covering at 420 °C. Remove the sample from the furnace and allow to cool. Weigh the beaker with sample again and record the weight (Y = weight of ashed sample - weight of beaker).
      6. Perform the following calculations:
         i) Loss on Ignition = X-Y
         ii) % Moisture = ((Sample Weight – X)/Sample Weight) x 100%
         iii) % Organic Matter = (Loss on Ignition/X) x 100%

2. Proximate and Ultimate Analysis
   NOTE: For proximate/ultimate analysis, four samples were analyzed: Low, High, Standard Fuel and High 2. PAH analysis was carried out on Low, High, and Standard Fuel. These were chosen as representative of the biochars produced since 2012.
   1. Conduct Proximate and Ultimate analyses at a commercial facility based on methods: ASTM D3172-13 [8] and D3176-09, Standard Practice for Proximate and Ultimate[9] Analysis of Coal and Coke, respectively.

3. pH
   1. Calibrate the pH probe daily before use with calibration standards.
   2. Add 0.25 g biochar to 25 ml distilled, deionized water.
   3. Shake manually for 2 min, then centrifuge for 3,000 x g for 5 min.
   4. Collect supernatant into glass test tube and measure pH.

4. Particle Size Distribution
   1. Analyze all samples in triplicate via progressive dry sieving adapted from ASTM D5158-98 [10] using seven U.S. Standard sieves and pan (4.7, 2.0, 1.0, 0.50, 0.25, 0.15, and 0.0075 mm)
      1. Record the weight of each empty sieve and stack the sieves in order from pan to 4.7 mm with the 4.7 mm sieve being at the top.
      2. Place 60 g of biochar in the 4.7 mm sieve, place the lid on top and secure the stack of sieves on the shaker.
      3. Shake for 10 min and record the weight of each sieve. Report the data in an excel file as percent remaining in each sieve.

3. Test Category B: Toxicant Reporting

1. Germination Tests
   1. Use the seed germination testing method outlined by Solaiman *et al.* (2012) [11].
      1. Use filter paper and potting soil as positive controls.
      2. Ensure that the respective weights of each treatment is 3 g of biochar, 10 g of potting soil, and 1 piece of filter paper.

NOTE: These values are based on volume in the Petri dish so that each dish is ~50% full (by volume).
3. Into the Petri dishes (8.5 cm in diameter), place five *Cucurbita pepo* spp. *pepo* (pumpkin) seeds and 50 *Medicago sativa* (alfalfa) seeds into each treatment.
4. Using a graduated cylinder add 15 ml of water to all Petri dishes, then cover them with their respective lids.
5. Place the Petri dishes for germination under a 14:10 hr (day:night) fluorescent photoperiod and maintain temperature at 27 ºC (±6 ºC).
6. After seven days record the number of seeds germinated. Report results as % germinated per Petri dish. Measure the root length of germinated seeds using a ruler. Report root lengths as a sum for each Petri dish (cm/Petri dish).

2. Earthworm Avoidance
   1. Store *Eisenia fetida* in a healthy soil matrix comprised of peat moss and potting soil and maintain soil moisture at ~30%.
   2. Use earthworm avoidance method described by Li *et al*. (2011). Choose worms ranging from 0.3–0.6 g in size.
      1. For this assay, use six avoidance wheels (**Figure 1**) or similar structure to those outlined in Environment Canada's Acute Avoidance Test (Environment Canada, 2004).
      2. Mix biochars separately using a spade and bucket with potting soil at a rate of 2.8% (by weight).
      3. Fill each of the six compartments with 120 g of soil or soil/biochar mixture, with every other compartment serving as an unamended control (**Figure 1**) *i.e.* soil without biochar. Add 10 worms to the round middle compartment.
      4. Expose the worms for 48 hr keeping the avoidance wheel covered with aluminum foil to prevent worm escape. Maintain temperature conditions for the avoidance wheels between 20–25 °C. Monitor the soil moisture and maintain at ~30%.
      5. After 48 hr remove the worms and record their location in the avoidance wheel, *i.e.* if they are in the i) amended or ii) unamended compartments. Do not reuse worms for future testing.

3. Polycyclic Aromatic Hydrocarbons (PAHs)
   1. Analyze PAHs by solvent extraction and GC-MS based on EPA 8270 [12].

4. Polychlorinated Biphenyls (PCB) Concentration
   1. Dry samples (10 g) overnight at 25 °C for 18–24 hr then grind them to a fine powder (particle size < 0.15 mm) with 10 g sodium sulphate and 10 g Ottawa sand.
   2. Include one analytical blank (Ottawa sand), one control (a known amount of PCB standard) and one analytical duplicate sample for every 10 unknown samples.
   3. Place 2 g sample into Soxhlet thimble and add 100 µl decachlorobiphenyl (DCBP) as an internal surrogate standard.
   4. Extract samples in a Soxhlet apparatus for 4 hr at 4–6 cycles per hour in 250 ml of dichloromethane.
   5. Using a gas chromatograph equipped with a micro-^{63}Ni electron capture detector (GC/µECD), a fused silica capillary column (30 m, 0.25 mm ID × 0.25 µm film thickness) and appropriate software analyze biochar extracts for total Aroclors. Use helium as the carrier gas at a flow rate of 1.6 ml/min. Use Nitrogen as the makeup gas for the electron capture detector (ECD). Report values as µg/g dry weight.

5. Metal Analysis
   1. Air-dry samples for 18–24 hr and grind into a fine powder (particle size < 0.15 mm) with a mortar and pestle.
   2. Using reagent grade concentrated acids, heat 0.5 g of the sample in 2 ml 70% (w/w) nitric acid and 6 ml 38% (w/w) hydrochloric acid, until the volume is reduced to 1–2 ml. Then make-up the solution to 25 ml in a volumetric flask using distilled, deionized water, filtered through a Whatman No. 40 filter paper.
   3. Analyze samples using a simultaneous inductively coupled plasma atomic emission spectrometer (ICP-AES) with the following standards/controls (see step 3.5.3.1). Analyze multi-element ICP standards and check % error and correlation coefficients of the calibration curves. Standards are purchased in custom blends with many elements in each standard. Each element has a 3 point calibration curve (for example cadmium is run at 0, 0.1, 1.0 and 5 ppm). Verify curves with calibration check standards. Recalibrate approximately every 18 samples.
      1. Add internal standards (indium and scandium) 'on line' with samples to verify instrument stability. Analyze samples with additional quality control standards including certified reference materials (Bush, Branches and Leaves; White Cabbage and Spinach), method blanks (add acids to an empty digestion tube and treat them as described in 3.5.2 above), analytical duplicates, and field duplicates.

6. Mercury
   1. Ensure the instrumentation meets the criteria outlined in US EPA Method 7473 and allows for direct mercury measurement
   2. Weigh 100 mg of ground air-dried biochar (particle size < 0.15 mm) into quartz or nickel weigh boats.
   3. Use an ICP-AES stock solution of 1,000 µg/ml Hg and 5% hydrochloric acid in double deionized water (DDI) to make working stocks (5 µg/ml, 1 µg/ml, 0.1 µg/ml, 0.01 µg/ml) and calibration standards.
   4. Use a cleaned empty boat as a method blank. Analyze samples starting with a Method blank, Low QC (20 ng Hg – 20 µl of 1 µg/ml Hg), Blank, High QC (200 ng Hg – 40 µl of 1 µg/ml Hg), Blank, Blank, Standard Reference Material (MESS-3), Blank, MESS-3, Blank, Sample 1, Blank, Sample 2, Blank, Sample 2 dup, Blank, Sample 3, Blank, *etc.*
   5. Place the boats in the instrument chamber where the sample will thermally decompose in a continuous flow of oxygen.
      NOTE: The combustion products will then be carried off in the oxygen flow and then further decomposed in a hot catalyst bed. Mercury vapors will be trapped on a gold amalgamator tube and subsequently desorbed for spectrophotometric quantitation at 254 nm.

4. Test Category C: Biochar Advanced Analysis and Soil Enhancement Properties

1. Ammonium as Nitrogen
   NOTE: The method makes use of the Berthelot reaction wherein ammonium salts in the solution react with phenoxide. Addition of sodium hypochlorite causes the formation of a green-colored compound. Sodium nitroprusside is added to intensify the color.
   1. Weigh 5 g of ground air-dried sample (particle size < 0.15 mm) into a 125-ml Erlenmeyer flask. Add 50 ml of 2 M (0.01% (V/V) KCl. Put the flasks on a rotating shaker for 1 hr at 200 rpm. After shaking is complete, filter the samples through Whatman No. 42 filter paper into 100-ml plastic vials.
   2. Prepare Reagent Solutions:
      1. Alkaline Phenol — measure 87 ml of liquefied phenol into 1-L volumetric filled 2/3 with DDI water. Add 34 g NaOH, make up to volume with DDI water.
      2. Hypochlorite Solution — using 100-ml graduated cylinder measure 31.5 ml of commercial bleach (5–10%) and fill to 100 ml with DDI water. Transfer to bottle and add 1.0 g of NaOH pellets and allow them to dissolve.
      3. EDTA solution — dissolve 32 g of di-sodium EDTA and 0.4 g NaOH in a 1-L volumetric filled 2/3 with DDI water. Add 0.18 g nitroprusside and dissolve by shaking. Make up to volume with DDI water and add 3 ml Triton (10%).
   3. Make calibration standards (0.1, 0.2, 0.3, 0.5, 1.0, and 2.0 µg/ml N Concentration) using reagent grade NH_4Cl and DDI water. Prepare QC reference standard from a reagent grade source of ammonium chloride different from the source used to make the standards. Use double deionized water as the blanks.
   4. Begin running the autoanalyzer. Design each run to start with the High Standard (2.0 µg/ml N) x 2, Calibration Standards (high to low), Method Blank, High Standard, Low Standard (0.1 µg/ml N) x 2, Wash Water, QC Reference Sample x 2, Samples, Sample duplicate, and High Standard., and Wash Water.
      NOTE: The autoanalyzer software will automatically calculate concentrations in the extract.
   5. Calculate the Biochar Concentration = (Extract Concentration x 50 ml (KCl)) / 5 g Biochar Sample.

2. KCl Extractable Nitrite and Nitrate by Autoanalyzer
   NOTE: The Griess Ilosvay colorimetric method utilizes the reaction of nitrite ions with sulfanilamide under acidic conditions to form a diazo compound. The compound further reacts with *N*-1-naphthylethylenediamine dihydrochloride to form a magenta azo dye. Nitrate in the sample is converted to nitrite through exposure to a reducing agent (in this case a copper-cadmium reducing column). This gives a measure of the nitrate + nitrite concentration in the sample.
   1. Weigh 5 g of ground air-dried sample (particle size < 0.15 mm) into 125-ml Erlenmeyer flask. Add 50 ml of 2 M (0.01% (V/V)) KCl. Put the flasks on a rotating shaker for 1 hr at 200 rpm. After shaking is complete, filter the samples through Whatman No. 42 filter paper into 100-ml plastic vials.
   2. Allow reagents (Ammonium chloride and Color Reagent) to warm to room temperature.
   3. Turn on colorimeter to let the lamp warm up. Stored within the auto analyzer are reagent lines labeled Ammonium chloride, Color Reagent and Water; start the pump and allow water to run through the system, check all pump-tubing lines for proper function.
   4. Once the system has equilibrated, place lines in the respective reagents and allow to run for 5–10 min. Turn on the chart recorder. Wait for baseline to stabilize, and set to the 10^{th} chart unit.
   5. Prepare 100 µg/ml nitrate and nitrite QC Stock Standards from KNO_3 and $NaNO_2$ and DDI water, respectively. To make a 10 µg/ml Intermediate Standard, add 5 ml of 100 µg/ml stock solution to 50-ml volumetric flask and make up to volume with 0.01% KCl. To make Calibration Standards combine 0.01% KCl and the 10 µg/ml intermediate standard prepared in 25-ml volumetric flasks to make calibration standards (0.05, 0.2, 0.5, 1.0, 1.5, 2 µg/ml NO_3 or NO_2). Use KCl for method blanks.
   6. Prepare spikes using 5 g of Ottawa sand (inert material) and add 0.05 ml of the appropriate 1,000 µg/ml QC standard for an end result of 10 mg N/kg sample. Make a combined NO_3 + NO_2 spike by spiking a single sample with 0.025 ml of each 1,000 µg/ml QC standard stock. Prepare one sample spike per run by spiking 5.0 g of the unknown biochar sample with 0.025 ml of the appropriate 1,000 µg/ml QC standard stock.
   7. Begin running analysis. Include a full set of calibration standards, two QC Reference Samples, at least two KCl blanks, and at least two Nitrite Standards, a set of Ottawa Sand Spikes and blanks and a Sample Spike in each run.
      NOTE: Standards may be rerun as markers between every 5 unknown samples and to verify the values for preparation of the standard curve.
   8. Repeat the 2.0 µg/ml standard at the end of each run. Run duplicate samples at a minimum rate of 10%. Run Nitrite + Nitrate analysis first, followed by the Nitrite analysis.
   9. Record on the Nitrite Nitrate Worksheet peak heights of all standards, QC checks and samples. Use the number of chart units as the measurement of height. To calibrate the instrumentation, use the relative heights of the standards. Ensure that the R^2 value lies above 0.99, if not re-run the standards.
   10. Calculate the concentration of the samples using the formula:
       Extract Concentration = (Peak Height - Intercept of the Calibration Curve/Calibration Curve Slope) x Dilution
       Biochar Concentration = (Extract Concentration x 50 ml (KCl)) / 5 g Biochar Sample
   11. Subtract the estimated nitrite concentration from the nitrate plus nitrite concentration to calculate nitrate.

3. Extractable Phosphorous (2% Formic Acid Extraction)
   NOTE: The auto analyzer software automatically calculates concentrations. The software reports calibration information, goodness of fit of the calibration curve, concentrations for all samples, calibrants, blanks and QC samples that have been run.
   1. Prior to analysis store samples in a clean glass container or sterile plastic bag. Keep samples refrigerated and analyze within two weeks or keep frozen for up to one year.
   2. Make all standards and QC standard with the same extraction fluid that is used for the samples. Use Estuarine Sediment as a standard reference material and in every bath of samples include two blanks to be extracted.
   3. Using a 1-L volumetric filled to 750 ml with DDI water, add 20 ml (98–99%) formic acid and fill to volume with DDI water.

4. Add 1.0 g of ground air-dried sample (particle size < 0.15 mm) into a 125-ml Erlenmeyer flask. Add 50 ml of 2% formic acid solution. Put the flasks on sonicator for 10 min, then transfer onto rotating shaker for 1 hr at 200 rpm. After shaking, filter samples using Whatman No. 42 filter paper into another set of 125-ml Erlenmeyer flasks.
5. Prepare Standards and Spikes:
    1. Prepare a 1,000 µg/ml QC Stock Standard from potassium dihydrogen orthophosphate and DDI water. Use the QC Stock Standard to make the Calibration Standards (5 µg/ml, 1 µg/ml, 0.5 µg/ml, 0.2 µg/ml, 0.1 µg/ml). Use 0.100 ml of the QC Standard to make the QC Spike. To make a QC Standard Check, add 0.100 ml of the QC Stock Standard to a 50-ml volumetric flask and make it up to volume with KCl.
    NOTE: This is a 0.2 µg/ml dilution concentration.
    2. Use Estuarine sediment as a QC Reference Sample. Use 0.01% KCl as the method blank.
6. Analyze on the autoanalyzer system. Set samples up as Primer (High Standard (0.5 µg/ml), Calibrants (5 µg/ml, 1 µg/ml, 0.5 µg/ml, 0.2 µg/ml, 0.1 µg/ml), Blank, Null, High Standard (0.5 µg/ml), Low Standard (0.1 µg/ml), Low Standard (0.1 µg/ml), Null, QC (Reference Sample/ Estuarine Sediment), QC (Reference Sample/Estuarine Sediment), Method Blank, Sample 1, Sample 2, Sample 2 Dup, Sample 3 *etc.*, High Standard, Null.
7. In every batch of samples also extract two blanks: one is a calibration blank and it is to be placed in the standard rack of the autosampler, the other is a method blank and it is to be placed in the sample tray.

4. Specific Surface Area
NOTE: Analysis for Brunauer-Emmett-Teller (BET) surface area was conducted in the Chemical Biological Radio Nuclear (CBRN) Protection Lab at RMC. The method utilizes N_2 gas sorption analysis at 77 K in a relative pressure range from 0.01 to 0.10 after degassing at 120 °C for a minimum of 2 hr. A duplicate sample was analyzed for every 6 unknown samples. Samples are not ground into powdered form prior to analysis.
NOTE: Degassing times and pressures are specific to instrument manufacturer and the method provided has been validated previously with high temperature activated carbons.
5. Cation Exchange Capacity (CEC)
    1. Follow the sodium acetate method for CEC described by Laird and Fleming (2008) to calculate CEC.
        1. Include one analytical blank (DDI water), standard reference material (Ottawa Sand) and duplicate for every 10 samples.
        2. Prepare saturating solution (1 M NaOAc pH 8.2) by dissolving 136.08 g of $NaOAC{\cdot}3H_2O$ in 750 ml distilled, deionized water. Adjust the pH to 8.2 by adding acetic acid or sodium hydroxide. Dilute to 1 L with DDI water.
        3. Prepare first rinsing solution (80% isopropanol (IPA)) by combining 800 ml IPA with 200 ml distilled, deionized water. Then prepare the second rinsing solution (100% IPA).
        4. Prepare the replacing solution (0.1 M NH_4Cl) by dissolving 5.35 g NH_4Cl into 1 L distilled, deionized water.
        5. Weigh 0.2 g of sample (air dried, not ground) into a 30-ml centrifuge tube. At the same time, weigh 0.5 g of the same air dried sample into a pre-weighed aluminum drying pan. Place the sample in the aluminum drying pan in the oven at 200 °C for 2 hr, cool it in a desiccator and then weigh again to determine the water content of the air-dried sample. Use this sample to calculate the water content correction factor, F (step 4.4.1.10).
        6. Add 15 ml of the saturating solution, vortex, then centrifuge at 3,000 x g for 5 min. Decant and carefully discard the supernatant to ensure no sample is lost. Repeat this step two more times.
        7. Add 15 ml of the first rinsing solution. Vortex and centrifuge at 3,000 x g for 5 min. Decant and carefully discard the supernatant. Repeat this step several times, each time measuring the electrical conductivity of the supernatant solution. When the conductivity of the supernatant drops below the conductivity of NaOAc saturated with IPA (~6 µS/cm), switch to the second rinsing solution. Continue to rinse the sample until the conductivity of the supernatant drops below 1 µS/cm.
        8. Allow the sample to air dry in a fume hood, then add 15 ml of the replacing solution. Vortex and centrifuge at 3,000 x g for 5 min. Decant and save the supernatant into a 100-ml volumetric flask. Repeat this step three more times, each time saving the supernatant into the same volumetric flask. Then bring the volumetric to 100 ml with distilled, deionized water.
        9. Analyze the sodium content via inductively coupled plasma-atomic emission spectrometry (ICP-AES) as previously described.
        10. Perform the following calculations:
        F = (weight of oven dried, air dried sample - weight of air dried sample)
        C = Na concentration (mg/L) in the 100-ml volumetric flask
        W = weight (g) of air-dry sample added to centrifuge tube
        CEC = (C x 0.435)/(W x F)(cmol/kg)

Representative Results

A summary of all results including a comparison to the criteria set by the IBI [13] can be found in **Tables 1** (summary), **2** (New, High, Low, Third Feedstock and High-2 biochars) and **3** (Old biochar). All biochars and feedstocks used in 2012 and 2013 (**Table 2**) were well within the criterion set by the IBI and there were little differences among biochars. Old biochar (**Table 3**), the first biochar submitted for testing, was made from used shipping pallets and construction wastes and was determined to have elevated levels of the metals arsenic, chromium, copper, and lead. Old biochar also had the lowest levels of organic carbon (63.2%) as determined by loss on ignition. This biochar had the highest levels of extractable phosphorus (850 mg/kg) and CEC (34.8 cmol/kg), as well as the highest percentage of fine particles (<0.5 mm, 48%). Old biochar was also the only biochar to fail the germination test (**Figure 3**) and it was determined that *Eisenia fetida* (soil invertebrate) significantly avoided the 2.8% Old biochar amendment, whereas they preferred the 2.8% amendment of the New biochar (**Figure 2**).

Test Category A: Basic Biochar Utility Properties

Biochar production via pyrolysis is essentially the carbonization of biomass. The carbonization process allows for the transformation of structured organic molecules of wood and cellulose materials into carbon, or carbon-containing residues, which are often aromatic in nature [14-18].

Carbonization is obtained through the elimination of water and volatile substances from the biomass feedstock, due to the action of heat during the pyrolysis process [19]. All of the biochars produced at the commercial greenhouse contained a relatively low moisture percentage (<5%) with the exception of Old biochar. All biochars are categorized by the IBI as Class A (>60%) in terms of their composition of organic carbon as a result of complete carbonization of the feedstock material via pyrolysis. Thus due to the high percentage of organic carbon, all biochars produced have a low percentage of ash (<2.5%), which is the inorganic or mineral component of the biochar [13]. Although these low ash biochars do not provide substantial amounts of nutrients directly to the soil as do their high-ash biochar (often made from manures and bones) counterparts; the carbon content of these biochars is much higher and therefore they have higher long-term nutrient retention abilities [20-22].

The hydrogen to carbon ratio (H:C) is a term often used to measure the degree of aromaticity and maturation of the biochar, which has been linked to their long-term stability in the environment [18]. For biomass feedstock containing cellulose and lignin, the H:C ratios are approximately 1.5. However, pyrolysis of these materials at temperatures greater than 400 °C is expected to produce biochars with H:C ratios <0.5. It has been reported that an H:C ratio < 0.1 indicates a graphite-like structure in the biochar [23]. All biochars in this report have H:C ratios less than 0.02, indicating that these biochars are highly aromatic in nature and will have long-term stability in the environment.

Soil pH is a measure of soil acidity, and unfortunately many agricultural soils in Canada and worldwide are acidic (pH < 7), meaning that they are not ideal for crop growth. Biochars with an alkaline pH (> 7), such as those being produced at the greenhouse, can be added to acidic soils to increase the soil pH to levels that are more appropriate for plant growth.

Another important soil characteristic for plant growth is particle size distribution (PSD). Biochars that have a higher percentage of coarse particles may favorably increase soil aeration and prevent biochar movement into the subsoil over time, thereby increasing the length of time biochar offers benefits to plant growth [24]. However, smaller particle sizes are favored for biochars that are being produced for remediation purposes with the intent to sorb contaminants and minimize their bioavailability, as contaminants are more easily able to access pore space for binding [3,25,26]. Also smaller particles sizes increases the number of biochar particles per unit volume of soil which is favorable for contaminant sorption[27]. As in a previous study[3], fine particles are defined as those < 0.25 mm and coarse particles as > 0.5 mm. The biochars named New-, High- and Third Feedstock have a high proportion of coarse particles (~98%), and a low proportion of fine particles (~2%). The biochar produced at a slightly lower temperature, had 89% coarse and 11% fine particles sizes. All of these biochars may offer substantial improvements to soil texture and aeration especially in degraded or clay type soils. The Old biochar had a PSD that differed substantially from the others, having 52% coarse and 48% fine particles. A biochar with this PSD may be preferable for use at contaminated sites, where contaminant sorption is the primary focus.

Test Category B: Toxicant Reporting

Biological testing of biochar is important to assess the toxicity (if any) of these materials to soil invertebrates and plants. To date, there is little existing literature on the potential impact of biochar on terrestrial organisms and their associated response, and often the literature that does exist presents conflicting results. Exposure to contaminants may inhibit earthworms ability to perform essential soil functions such as decomposition, nutrient mineralization, and soil structure improvements [28]. New biochar showed no detrimental effects on the earthworm *Eisenia fetida* as assessed by earthworm avoidance, however worms significantly avoided Old biochar (**Figure 2**). Germination assays are a technique used to evaluate the toxicity of a particular material to plants. Potting soil served as a better control than filter paper as the filter paper often encouraged mold formation. Pumpkin and alfalfa seeds germinated well with 67% ± 12% and 81% ± 6% germination, respectively. Roots also proliferated well with average lengths after seven days being 14 cm ± 0.6 cm and 55 cm ± 8 cm for pumpkins and alfalfa, respectively. As with the earthworm avoidance studies Old biochar showed toxicity to plants and all other biochars evaluated showed no detrimental effects to seed germination as measured by percent germination and root length after seven days (**Figure 3**).

Although some types of biochar have the potential to sorb organic contaminants and reduce their toxicity in the environment, careful characterization of the biochar is required to ensure that it does not contain harmful contaminants such as PAHs, PCBs, and metals as a result of contaminated feedstocks or pyrolysis conditions. None of the biochars produced at the greenhouse had PAH concentrations exceeding IBI guidelines. Old biochar was determined to have elevated levels of PCBs and the metals arsenic, chromium, copper, and lead, however none of the biochars produced from the other two biomass materials contained metals above IBI guidelines. Old biochar was produced from used shipping pallets and construction wastes which is likely the source of the metal contamination. Although Old biochar would not be suitable for use in agricultural soils or home gardens, all other biochars could be used for these purposes.

Test Category C: Biochar Advanced Analysis and Soil Enhancement Properties

Biochars containing a high concentration of ammonium and nitrate may be applied to agricultural soils to offset the requirements for synthetic fertilizers. However, if biochar contains an excess of these nitrogen compounds then application on a large scale could increase the atmospheric N_2O concentration and contaminate drinking water sources with nitrates. None of the biochars studied contained elevated amounts of ammonium or nitrate.

Phosphorus is an essential component for many physiological processes related to proper energy utilization in both plants and animals. Biochars with moderate amounts of available phosphorus will act as important plant fertilizers. In Ontario, soils containing 15–30 mg/kg phosphorus are considered low, 31–60 mg/kg moderate, and 61–100 mg/kg high. Old biochar was highest in available phosphorus at 850 mg/kg and may not be suitable for adding to soils already classified as high in phosphorus. However, all other biochars tested had a much lower amount of available phosphorus and would not be expected to cause problems when added at rates up to 10% (w/w).

The components of biochar (except moisture) that are released during pyrolysis are referred to as volatile matter. These components are typically a mix of short and long chain hydrocarbons, aromatic hydrocarbons with minor amounts of sulfur. Volatile matter was determined via proximate analysis which also determines the moisture and ash content of biochars (section 2.2). The volatile content affects the stability of the material [29], N availability and plant growth [30]. In theory, biochars high in volatile matter are less stable and have a higher proportion of labile carbon that provides energy for microbial growth and limits the availability of nitrogen necessary for plant growth. A study by Deenik *et al.,* (2010) considered 35% volatile matter to be high (inducing nitrogen deficiency), and 10% volatile matter to be low. All biochar in this report contained

less than 20% volatile matter, and hence would not be expected to limit plant growth. Proximate analysis determination of volatile matter is most important for biochars with low ash concentrations such as those produced at the commercial greenhouse.

Specific surface area (SSA) is a measure of the porosity of a biochar. It includes not only the external biochar surface area, but also the surface area within the pore spaces and is an important characteristic used to predict the ability of a biochar to sorb organic contaminants. Contaminant sorption has been attributed to π-π interactions (attractive, non-covalent binding) between the aromatic ring(s) of the contaminant and those of the biochar [31]. Activated carbon (AC) is a charcoal-like material that is treated during its production to maximize its porosity and therefore has higher SSAs than most biochars. Although all the of biochars presented in this report have SSAs in the 300 m^2/g range (*i.e.* much less than that of AC; ~800 m^2/g), as reported in Denyes *et al.*, 2012 and 2013, the biochars, Old and New, have both shown significant potential to serve as a soil amendment for the remediation of PCBs.

Cation exchange capacity (CEC) is a measure of the number of cations (positively charged ions) that a soil particle is capable of holding at a given pH. The ability of the soil to hold cations is due to electrostatic interactions with negatively charged sites on the surface of a particle, such as hydroxyl (OH^-) and carboxyl (COO^-) groups [32, 33]. The CEC of the soil can be linked to the ability of the soil to hold nutrients and retain cations from fertilizers which are essential for plant growth. Also, many environmental contaminants such as lead, cadmium and zinc have positive charges; therefore soils with a high CEC may function to prevent the leaching of these contaminants into drinking water sources. Biochars have been reported to increase the CEC of soils, due to the slow oxidation of the biochar surface which increases the number of negatively charged sites, and therefore may reduce fertilizer requirements and immobilize positively charged contaminants in soils [32]. Typically, sandy soils have a CEC between 1–5 cmol/kg, loam soils 5–15 cmol/kg, clay type soils >30 cmol/kg and organic matter 200–400 cmol/kg. The methods for determining the CEC of biochar are still in their infancy and therefore should be considered in relative terms. The CEC of the biochars produced at the greenhouse are higher than the CEC of PCB-contaminated soils (Denyes *et al*., 2012), but lower than compost amended soils.

Figure 1. Earthworm avoidance wheel. The wheels are produced from steel and the worms are allowed to move throughout the compartments via multiple holes which are approximately 5 cm in diameter.

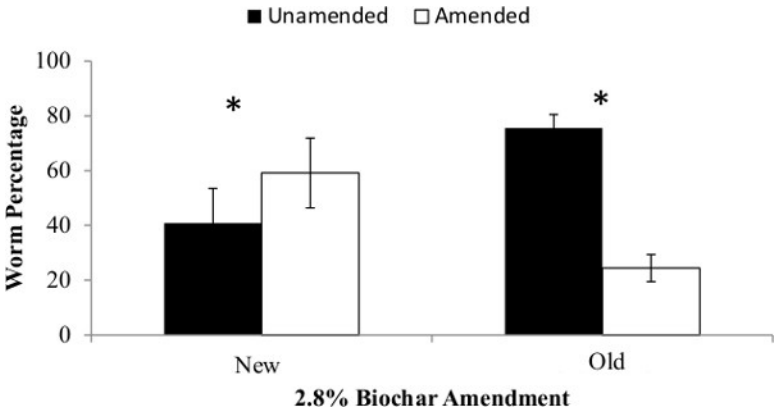

Figure 2. Earthworm avoidance assay of Old and New type biochars. The biochar titled "Old" was produced via construction wastes, whereas the biochar titles "New" was produced from sawdust materials. * indicates a significant difference between unamended potting soil and potting soil amended with 2.8% of either biochar ($p < 0.05$).

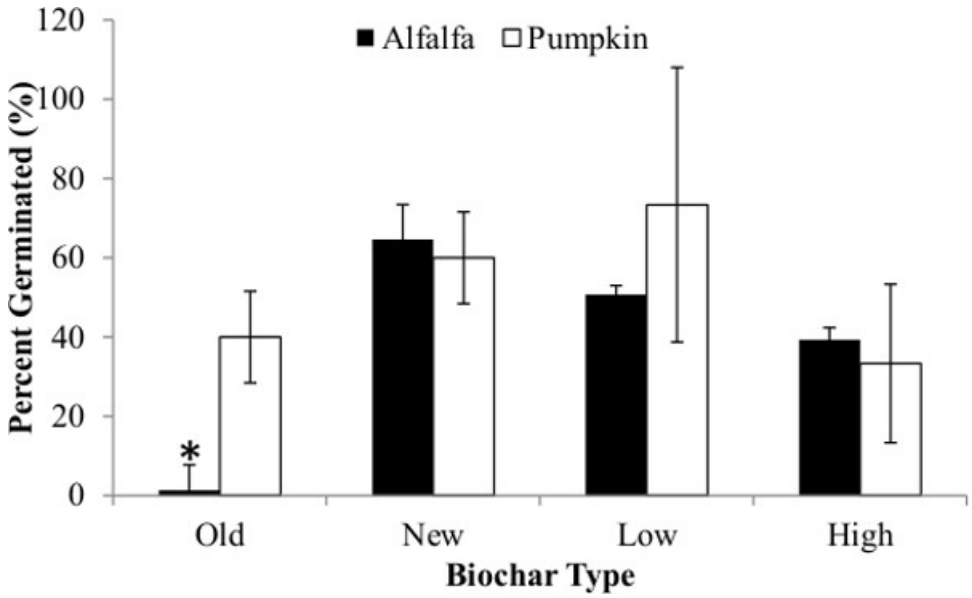

Figure 3. Percent germination of two different plant species. Pumpkin (*Cucurbita pepo* spp. *pepo*) and alfalfa (*Medicago sativa*) were grown in triplicate in various biochars produced at a commercial greenhouse for seven days. Old and New refer to biochars made from different feedstocks, whereas Low and High refer to different temperatures of pyrolysis. * indicates significantly difference from the controls (potting soil and filter paper).

Sample	Feedstock	Pyrolysis Temperature	Organic Matter (LOI)	pH	CEC	PSD	PSD	SSA
						Coarse	Fine	
		°C	%		cmol/kg	%	%	m^2/g
Old	1	>700	63.2	9.3	34.8	51.7	48.3	373.6
New	2	700	97.8	9	16	98.7	1.3	324.6
Low Temp	2	500	96.7	8.7	15.9	86.2	13.8	336.9
High Temp	2	>700	97.9	8.4	11.1	98.1	1.9	419.5
Third Feedstock	3	700	96.2	9.6	13.2	97.6	2.4	244.4
High Temp-2	3	>700	97.1	9.1	17.1	97.9	1.9	428
LOI: Loss on Ignition, CEC: Cation Exchange Capacity, PSD: Particle Size Distribution, SSA: Specific Surface Area								

Table 1. Feedstock type, pyrolysis temperature and physical characteristics of the six biochars.

Requirement	IBI	Biochar	Feedstock Range	Unit	
	Criteria	Range			
Test Category A: Basic Biochar Utility Properties - Required for All Biochars					
Moisture	Declaration	<0.1–4.3		%	
Organic Carbon	Class 1 > 60%	96.2–97.8 (LOI)		%	
	Class 2 > 30%	92.44–97.93(Pro/Ult)			
	Class 3 > 10 < 30%				
H:C_{org}	0.7 max	0.01–0.02		Ratio	
Total Ash	Declaration	1.38–2.26		%	
Total N	Declaration	0.28–1.06		%	
pH	Declaration	8.4–9.6		pH	
Particle Size Distribution	Declaration	86–98		% Coarse	
		1.3–14		%	
				Fine	
Test Category B: Toxicant Reporting- Required for All Feedstocks					
Germination	Pass/Fail	Pass			
Earthworm Avoidance	Declaration	No Avoidance			
Polyaromatic Hydrocarbons (PAHs)	6–20	<2.0		mg/kg	
Polychlorinated Biphenyls (PCBs)	0.2–0.5	<0.1		mg/kg	
Arsenic	12–100	<1.0	<1.0	mg/kg	
Cadmium	1.4–39	<1.0	<1.0	mg/kg	
Chromium	64–1,200	<2.0	<2.0–2.6	mg/kg	
Cobalt	40–150	<1.0	<1.0	mg/kg	
Copper	63–1,500	3.6-6.5	<2.0–5.9	mg/kg	
Lead	70–500	<2.0–2.7	<2.0–8.1	mg/kg	
Mercury	1,000–17,000	<5.0–294		ng/g	
Molybdenum	5–20	<2.0	<2.0	mg/kg	
Selenium	1–36	<10	<10	mg/kg	
Zinc	200–7,000	5.6–56.2	7.8–30.5	mg/kg	
Chlorine	Declaration			mg/kg	
Sodium	Declaration	137-878	<75-770	mg/kg	
Test Category C: Biochar Advanced Analysis and Soil Enhancement Properties- Optional for All Biochars					
Mineral N (Ammonium and Nitrate)	Declaration	<0.2–6.1		mg/kg	
Total Phosphorus	Declaration	69.5–276	52.5–74	mg/kg	
Available Phosphorus	Declaration	9–80		mg/kg	
Volatile Matter	Declaration	12.47–19.09		%	
Specific Surface Area	Declaration	244–428		m^2/g	
Cation Exchange Capacity	Declaration	11.1–17.1		cmol/kg	

Table 2. Summary Criteria and Characteristics for New, High, Low, Third and High-2 Biochars and Feedstocks. All biochars listed in this table are produced from similar feedstocks at the same pyrolysis facility.

Requirement	IBI	Biochar Range	Feedstock Range	Unit
	Criteria			
Test Category A- Basic Biochar Utility Properties - Required for All Biochars				
Moisture	Declaration	20		%
Organic Carbon	Class 1 > 60%	63.2 (LOI)		%
	Class 2 > 30%			
	Class 3 > 10 < 30%			
$H:C_{org}$	0.7 max			Ratio
Total Ash	Declaration			%
Total N	Declaration			%
pH	Declaration	9.3		pH
Particle Size Distribution	Declaration	52		% Coarse
		48		% Fine
Test Category B: Toxicant Reporting- Required for All Feedstocks				
Germination	Pass/Fail	Fail		
Earthworm Avoidance	Declaration	Avoided		
Polyaromatic Hydrocarbons (PAHs)	6–20			mg/kg
Polychlorinated Biphenyls (PCBs)	0.2–0.5	1.2		mg/kg
Arsenic	12–100	167	<1.0	mg/kg
Cadmium	1.4–39	<1.0	<1.0	mg/kg
Chromium	64–1,200	206	<20	mg/kg
Cobalt	40–150	5.3	<5.0	mg/kg
Copper	63–1,500	558	<5.0	mg/kg
Lead	70–500	314	<10	mg/kg
Mercury	1,000–17,000	<5.0		ng/g
Molybdenum	5–20	<2.0	<2.0	mg/kg
Selenium	1–36	<10	<10	mg/kg
Zinc	200–7,000	498	<15	mg/kg
Chlorine	Declaration			mg/kg
Sodium	Declaration	6460	<75	mg/kg
Test Category C: Biochar Advanced Analysis and Soil Enhancement Properties- Optional for All Biochars				
Mineral N (Ammonium and Nitrate)	Declaration	2.6		mg/kg
Total Phosphorus	Declaration			mg/kg
Available Phosphorus	Declaration	850		mg/kg
Volatile Matter	Declaration			%
Specific Surface Area	Declaration	373.6		m^2/g
Cation Exchange Capacity	Declaration	34.8		cmol/kg

Table 3. Summary Criteria and Characteristics for Old Biochar and Feedstock. The biochar listed in this table was produced from construction wastes at the same pyrolysis facility as the biochars listed in **Table 2**.

Discussion

All of the methods listed in the protocol have been carefully validated and extensively used for soils. As biochar characterization is still in its infancy, the effectiveness of these methods for the carbon-rich substrate was largely unknown. Hence, although these methods themselves are not novel, their application to routinely characterize biochar is. In terms of quality assurance/ quality control, there were no issues among any of the methods with respect to the blanks being below detection limits or the recoveries being correct for the standard reference materials. This

indicates that these methods are suitable to be used for the characterization of biochar and other charcoal-like materials. Many different methods have been used to characterize biochars in the literature[20, 34-41] however, as biochar becomes increasingly accepted as a soil additive, routine methods are required.

Cation exchange capacity was the only method in which difficulty arose. The method for calculating the CEC of a sample is dependent on the weight of sample and the concentration of sodium in that given weight. Biochar has a very low density and therefore does not pelletize at the bottom of the tube after centrifugation, as soil does. Therefore, when decanting and discarding the supernatant in steps 6 and 7 of the method (4.4), it is important to not lose any of the biochar sample. Pipetting the solution from the centrifuge was required to avoid any sample loss.

Other analytical methods were easily adapted from soil methods. Ultimate and proximate analysis is specific to biochar and similar products such as coal, and hence is not normally available in laboratories which routinely analyze soils. Another method (ASTM D1762) is available, for the determination of moisture, volatile matter, and ash in charcoal made specifically from wood. This method would also have also been suitable for proximate analysis. When determining loss on ignition for percent organic matter and percent moisture some may choose to perform these analyses at temperatures greater than 420 °C, especially if the biochars in question are produced via very high temperatures of pyrolysis. In the case this particular study 420 °C was sufficient to completely ash all biochars, and although not discussed this temperature was sufficiently high to ash even activated carbon.

Working with biological organisms such as plants and worms can often be challenging. Selecting the appropriate study organisms is of particular importance. The soil invertebrate *Eisenia fetida* is used frequently as a terrestrial organism model in contamination experiments because this species is capable of surviving at high concentrations of organic contaminants, is very well researched, and is ecologically relevant in many areas of the globe [2, 28, 42-46]. Soil invertebrates play an important role in the soil matrix, as they degrade organic matter, cycle nutrients, and transfer water. The plant species' alfalfa (*M. sativa*) and pumpkin (*C. pepo*) were chosen for the germination assays as they are commonly grown in Canada and have been used in our complimentary work on contaminant remediation [2, 3, 47]. Greenhouse conditions for germinating seeds need to be carefully monitored to ensure proper functioning of lighting and to avoid extreme temperature fluctuations.

The characterization of biochar is essential to its successful application as measured parameters will indicate the effectiveness of different biochars for different applications (*i.e.* whether a biochar is appropriate for contaminant sequestration, soil quality improvement, contaminant remediation *etc.*). Because the methods detailed here are widely available for soil analysis, they are a cost-effective means for characterization of biochars, and should be widely employed prior to large-scale application of biochar in the field.

Disclosures

The authors declare that they have no competing financial interests.

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