FINAL SCRIPT: APPROVED FOR FILMING

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Title: Assessment of Waste-Derived Biochars on the Health and Biological Activity of Soil

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No.**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No.**

3. Filming location: Will the filming need to take place in multiple locations? **Yes.**

If **Yes**, how far apart are the locations? The centrifuge is placed in another lab, but in the same faculty. It is 2 minutes away from the main filming location.

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No.**

Current Protocol Length
Number of Steps: 25
Number of Shots: 54

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Lenka Wimmerova:** We study how different biochars affect soil health. We test their impact on microbes, plants, and invertebrates to understand benefits and possible risks.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What are the most recent developments in your field of research?

1.2. **Lenka Wimmerova:** Researchers now focus on biochars from diverse wastes. There is also more focus on long-term field experiments, beyond standard laboratory testing.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

What technologies are currently used to advance research in your field?

1.3. **Anshu Shaw:** There are advanced technologies like DNA sequencing for microbes, soil structure imaging, and sensors for monitoring moisture, gases, and nutrients related to biochars.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.1*

What are the current experimental challenges?

1.4. **Anshu Shaw:** Biochars are never the same. Their properties change with feedstock and temperature, which makes it challenging to standardize experiments or link to field conditions.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.1.1*

What significant findings have you established in your field?

1.5. **Rafaella Denissa Muscalu:** Our study shows that biochar effects depend strongly on the feedstock type and the organism tested upon. Some stimulate microbes and plants, while others were toxic to soil organisms.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.1*

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

Videographer's Note: Some slate are missing in the shots. When you see repeated slates, it is for a change of framing (e.g to Closeup)

2 shots require masking:
1. DSCF3964 – freeze the display of the scale the moment number reached "7.437"
2. DSCF3993 - freeze the display of the scale the moment number reached "7.6"

During PH measurements, numbers at the scale were changing constantly, and for the shot it had to be still.

DSCF3984 – could not get a macro, so I did 4k for a zoom.

2. Preparation and pH Measurement of Soil-Biochar Mixtures

Demonstrator: Anshu Shaw

2.1. To begin, obtain the topsoil collected from the experimental field **[1]**.

2.1.1. WIDE: Talent placing the container with soil on the work bench.

Videographer's Note: Slate number is DSCF3928

2.2. Using gloved hands, manually remove visible surface debris such as plant material or stones **[1]** and sieve the soil to a particle size of less than 5 millimeters to eliminate finer debris **[2]**.

2.2.1. Talent removing debris by hand from a tray of collected soil.
Videographer's Note: Slate number is DSCF39232

2.2.2. Talent pouring soil into a sieve and shaking or tapping to collect finer particles below.
Videographer's Note: Slate number is DSCF3939

2.3. Now, weigh 100 grams of the sieved soil using a digital balance **[1]** and transfer it into labeled test containers **[2]**.

2.3.1. Talent weighing sieved soil on a digital balance until the display reads 100 grams.

2.3.2. Talent pouring 100 grams of soil into separate plastic test containers.

2.4. Add 1 gram, 5 grams, or 10 grams of each biochar type per 100 grams of soil to prepare 1, 5, and 10 percent soil-biochar amendments on a weight-to-weight basis **[1]**.

2.4.1. Talent measuring biochar on a balance and adding it to a container with 100 grams of soil.
Videographer's Note: Slate number is DSCF3940

2.5. Next, add distilled water to each container to reach 60 percent of the soil's maximum water holding capacity **[1]** and mix the contents of each container with a spatula to ensure uniform distribution of soil, biochar, and water **[2]**.

2.5.1. Talent using a pipette or measuring cylinder to add distilled water into each test container.
Videographer's Note: Slate number is DSCF3951

2.5.2. Talent using a metal spatula to mix the contents in each container, showing even blending.

2.6. Weigh each container after mixing and record the weight **[1]**. Cover the containers with parafilm to minimize evaporation **[2]**.

2.6.1. Talent placing each test container on a digital balance and recording the displayed weight.

2.6.2. Talent sealing the mouth of each container with stretched parafilm.

2.7. Then, place the containers in an incubator set at 27 degrees Celsius for 10 days **[1]**.

2.7.1. Talent placing the parafilm-covered containers into a temperature-controlled incubator.

2.8. For pH measurement, weigh 10 grams of soil-biochar mixture into a 50-milliliter conical tube using a balance **[1]** and add 25 milliliters of distilled water to the tube **[2]**. Shake the tube using a vortex mixer at medium speed **[3]** and filter the contents using Whatman number 1 filter paper into a clean 100 milliliter beaker **[4]**.

2.8.1. Talent measuring out 10 grams of soil-biochar mixture and transferring it into labeled 50 milliliter conical tube.

2.8.2. Talent pouring 25 milliliters of distilled water into the conical tube using a measuring cylinder.

2.8.3. Talent manually mixing the contents by tube inversion.

2.8.4. Talent pouring the mixture onto Whatman number 1 filter paper.
Videographer's Note: Slate number is DSCF3961

2.9. Now, measure the pH of the filtrate at room temperature between 20 and 25 degrees Celsius using a calibrated pH meter **[1]**.

2.9.1. Talent dipping the electrode of a digital pH meter into the beaker and reading the value displayed on the device.

3. Phytotoxicity Test with the Soil Sample
Demonstrator: Rafaella Denissa Muscalu

3.1. Add 20 grams of the incubated soil-biochar mixture into a clean 9-centimeter Petri dish **[1]**. Using a clean spatula, gently flatten the soil surface to create an even layer **[2]**. Prepare three replicates for each biochar concentration and set up two unamended control dishes **[3]**.

3.1.1. Talent spooning 20 grams of the soil-biochar mixture into an open Petri dish.

3.1.2. Talent using a clean spatula to gently level the soil surface inside the Petri dish.

3.1.3. Talent labeling and arranging multiple Petri dishes.

3.2. Place 10 *Sinapis alba* seeds evenly spaced on the surface of the soil in the Petri dish **[1]** and let the seeds rest naturally on the surface without pressing them into the soil **[2]**.

3.2.1. Talent carefully placing 10 seeds, spaced evenly, on top of the soil in each dish.

3.2.2. Close-up shot showing seeds sitting gently on the soil surface without being embedded.

3.3. Cover the Petri dish with its lid **[1]** and place it in an incubator set at around 25 degrees Celsius for 5 days, avoiding direct light **[2]**.

3.3.1. Talent placing lid on the Petri dish after seed placement.

3.3.2. Talent loading the covered dish into an incubator.

3.4. After the incubation period, measure the root length of each germinated seed from the seed edge to the tip of the root using a plastic ruler **[1]**. For cracked seeds with no elongation, record the root length as 0.1 centimeter **[2]**.

3.4.1. Talent using a transparent plastic ruler to measure the root length of sprouted seedlings in a Petri dish.

3.4.2. Close-up shot of a seed that has just cracked.

3.5. Calculate the inhibition effect percentage using the formula **[1]**.

3.5.1. TEXT ON PLAIN BACKGROUND:

$$\text{Inhibition effect (\%)} = \frac{\text{RLc} - \text{RLt}}{\text{RLc}} \times 100$$

Where:

RLc – average root length of seeds in control dish

RLt – average root length of seeds in the test dish.

4. Determination of Dry Matter (DM) and Dehydrogenase Activity (DHA) Test
Demonstrator: Anshu Shaw

4.1. Weigh 10 grams of soil into a heat-resistant glass or porcelain bowl using an analytical balance **[1]** and place the bowl in a drying oven preheated to 105 degrees Celsius for 2 hours **[2]**.

4.1.1. Talent placing a bowl on an analytical balance and adding soil.

4.1.2. Talent opening a drying oven, placing the soil-filled bowl on a rack inside.

4.2. After drying, transfer the bowl to a desiccator and let it cool for 10 minutes to prevent moisture absorption from the air **[1]**. Once cooled, weigh the bowl again using an analytical balance **[2-TXT]**.

4.2.1. Talent using tongs to transfer the hot bowl from the oven into a desiccator and sealing the lid.

4.2.2. Talent placing the bowl back on the balance and recording the new weight in a notebook. **TXT: Repeat this drying and weighing procedure for at least 3 soil samples**

4.3. Calculate the dry matter percentage using the formula **[1]**.

4.3.1. TEXT ON PLAIN BACKGROUND:

4.3.2. $\text{DM (\%)} = \frac{\text{Md}}{\text{Mw}} \times 100$

Where:

DM – content of dry matter (%)

Md – dry sample weight in g

Mw – undried (original) sample weight in g

4.4. For the DHA test, dissolve 12.12 grams of TRIS *(tris)* in 800 milliliters of distilled water to prepare a 100 millimolar buffer solution **[1-TXT]**. Adjust the pH to 7.6 using 1 molar hydrochloric acid **[2]**. Bring the total volume to 1 liter with distilled water **[3]** and store the solution at 4 degrees Celsius for use within one week **[4]**.

4.4.1. Talent adding 12.12 grams of TRIS powder into a beaker containing 800 milliliters of distilled water and stirring. **TXT: DHA: Dehydrogenase Activity**

4.4.2. Talent using a pH meter while adding 1 molar hydrochloric acid dropwise to adjust pH to 7.6.

4.4.3. Talent pouring distilled water into the beaker to bring the total volume up to 1 liter.

4.4.4. Talent placing the bottle in a refrigerator.

4.5. Next, dissolve 1 gram of TTC in 10 milliliters of the prepared TRIS buffer to make a 300 millimolar TTC substrate solution **[1-TXT]**. Store it in the dark at 4 degrees Celsius and use it within one week **[2]**.

4.5.1. Talent dissolving 1 gram of TTC in 10 milliliters of TRIS buffer and mixing until fully dissolved. **TXT: TTC: Triphenyltetrazolium Chloride**
Videographer's Note: Slate number is DSCF4003

4.5.2. Talent wrapping the container in foil and placing it in the refrigerator.

4.6. Add 100 milligrams of TPF to 10 milliliters of 96 percent ethanol and stir until fully dissolved to obtain a 33 millimolar TPF stock solution **[1-TXT]**. Dilute 0.5 milliliters of the stock with 50 milliliters of ethanol to prepare a 330 nanomolar per milliliter working solution **[2]**.

4.6.1. Talent weighing 100 milligrams of TPF and adding it to 10 milliliters of ethanol, followed by stirring. **TXT: TPF: Triphenyl Formazan**

4.6.2. Talent pipetting 0.5 milliliters of the stock into a flask containng 50 ml ethanol.

4.7. Then, prepare working standards of 0, 0.1, 0.2, 0.5, and 1 milliliters from the TPF working solution, and dilute each to a constant volume of 3 milliliters with ethanol **[1]**. Measure the absorbance of these standards at 485 nanometers to generate the calibration curve **[2]**.

4.7.1. Talent pipetting various volumes of working solution into labeled tubes kept in series.

4.7.2. Talent placing the sample in a spectrophotometer.

4.8. Now, weigh 5 grams of soil-biochar mixture into a 50-milliliter conical tube **[1]** and add 4 milliliters of TRIS buffer and 1 milliliter of TTC solution to each test sample **[2]**. For control sample, add only 4 milliliters of TRIS buffer **[3]**. Mix the tube gently by manual inversion **[4]** and incubate at 25 degrees Celsius in the dark for 6 hours **[5]**.

4.8.1. Talent weighing 5 grams of soil-biochar mixture and transferring into conical tube.

4.8.2. Talent pipetting 4 milliliters of TRIS buffer and 1 milliliter of TTC into test tube.

4.8.3. Talent labeling a tube as “Control”.

4.8.4. Talent sealing tube and gently inverting it by hand.

4.8.5. Talent placing the foil covered tube into an incubator set at 25 degrees Celsius.

4.9. After incubation, add 25 milliliters of ethanol to the tube **[1]** and place it on an orbital shaker at 250 revolutions per minute in the dark at 25 degrees Celsius for 1 hour **[2]**. After shaking, add 1 milliliter of TTC to the control tube **[3]** and centrifuge all the tubes at 2,000 *g* for 5 minutes at 25 degrees Celsius **[4]**.

4.9.1. Talent pipetting 25 milliliters of ethanol into a sample tube.

4.9.2. Talent loading the tube onto an orbital shaker, closing the lid.

4.9.3. Talent adding 1 milliliter of TTC the control tube.

4.9.4. Talent loading tubes into a centrifuge.

4.10. Transfer the resulting supernatants into clean cuvettes for analysis **[1]** and measure absorbance at 485 nanometers using a spectrophotometer **[2]**.

4.10.1. Talent using a pipette to transfer clear supernatant from centrifuged tube into a cuvette.

4.10.2. Shot of the spectrophotometer screen displaying absorbance readings at 485 nanometers.

4.11. Finally, use the calibration curve to determine the concentration of TPF in each test and control sample in nanomoles per milliliter **[1]** and calculate the dehydrogenase activity using the formula **[2]**.

4.11.1. Talent working at a computer station and performing calculations.
Videographer's Note: Slate number is DSCF4054

4.11.2. TEXT ON PLAIN BACKGROUND:

$$A = \frac{(Cs - Cb) \times V}{m \times DM \times RT}$$

Where:
A – enzymatic activity in mU g^{-1} (or nmol min^{-1} g^{-1}) of dry soil
Cs – concentration of TPF in test samples in nmol mL^{-1}
Cb – concentration of TPF in control sample in nmol mL^{-1}
V – total reaction volume (sum of substrate, buffer solution, and ethanol volumes, in mL)
RT – duration of the reaction in minutes
M – soil mass per tube in g
DM – dry matter content of soil (%)

Results

5. Results

5.1. Calcium and potassium content were highest in cigarette-butt and spent-hops biochars **[1]**, while spruce-wood biochar showed the highest levels of aluminum and iron **[2]**. Copper and magnesium were also strongly enriched in spent-hops biochar **[3]**.

5.1.1. LAB MEDIA: Table 2. *Video editor: Highlight the calcium and potassium values for "cigarette-butt" and "spent-hops*".

5.1.2. LAB MEDIA: Table 2. *Video editor: Highlight the aluminum and iron values for "spruce-wood"*.

5.1.3. LAB MEDIA: Table 2. *Video editor: Highlight the copper and magnesium values for "spent-hops"*.

5.2. Soil pH increased over time for all treatments **[1]**, with the cigarette-butt biochar at 10% weight by weight reaching the highest pH of 9.48 on day 15 **[2]** and hops biochar showing a 16.57% increase, with a maximum of 9.2 at 10% weight by weight **[3]**.

5.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight bars for day 10 and day 15 across*.

5.2.2. LAB MEDIA: Figure 2. *Video editor: Highlight the bar for "cigarette-butt " on day 15*.

5.2.3. LAB MEDIA: Figure 2. *Video editor: Highlight the bar for "hops biochar" on day 15*.

5.3. Water retention was highest in the control, which lost only 3.67% of its weight over 15 days **[1]**. Among biochars, cigarette-butt showed the least weight loss at 5.89% **[2]**, while coffee-grounds biochar exhibited the highest loss at 16.56% **[3]**.

5.3.1. LAB MEDIA: Figure 3. *Video editor: Highlight the control bar at day 15*.

5.3.2. LAB MEDIA: Figure 3. *Video editor: Highlight the "cigarette-butt" bar on day 15*

5.3.3. LAB MEDIA: Figure 3. *Video editor: Highlight the "coffee-grounds" bar on day 15*.

5.4. The *Enchytraeus albidus* population increased by 60% in the control soil **[1]**, but showed 53% inhibition with spruce-wood biochar **[2]**. Coffee-grounds and hops biochars both resulted in 20% inhibition **[3]**, while cigarette-butt biochar led to a 33% increase in worm reproduction **[4]**.

5.4.1. LAB MEDIA: Figure 4. *Video editor: Highlight the difference 'final' bar for the "control".*

5.4.2. LAB MEDIA: Figure 5. *Video editor: Highlight the "spruce-wood" bar*

5.4.3. LAB MEDIA: Figure 5. *Video editor: Highlight the bars for "coffee-grounds" and "hops"* .

5.4.4. LAB MEDIA: Figure 5. *Video editor: Highlight the "cigarette-butt" bar* .

5.5. Root elongation in *Sinapis alba* was stimulated by coffee-grounds biochar at 1% weight by weight by 79.16% **[1]**, while the same biochar inhibited growth by 47.08% at 5% **[2].** Spruce-wood biochar promoted root growth at all concentrations, peaking at 206.66% at 10% **[3]**.

5.5.1. LAB MEDIA: Figure 6. *Video editor: Highlight the "coffee-grounds" bar at 1% w/w.*

5.5.2. LAB MEDIA: Figure 6*. Video editor: Highlight the "coffee-grounds" bar at 5% w/w*

5.5.3. LAB MEDIA: Figure 6. *Video editor: Highlight the "spruce-wood" bar at 10% w/w*

5.6. Bacterial colony counts were highest for cigarette-butt biochar at 10% **[1]**, followed by spruce-wood biochar at 1% weight by weight **[2].** Coffee-grounds and hops biochars showed moderate stimulation at higher concentrations **[3]**.

5.6.1. LAB MEDIA: Figure 7. *Video editor: Highlight the bar for "cigarette-butt 10%"*

5.6.2. LAB MEDIA: Figure 7. *Video editor: Highlight the bar for "spruce-wood 1%"*

5.6.3. LAB MEDIA: Figure 7. *Video editor: Highlight the bars for "coffee-grounds" and "hops"*

5.7. Dehydrogenase activity was highest in coffee-grounds biochar at 1% weight by weight with around 3.53×10^{-3} milliunits per gram **[1]**, while spruce-wood biochar exhibited negative values at all concentrations **[2]**. Hops and cigarette-butt biochars showed decreased activity with increasing concentration **[3]**.

5.7.1. LAB MEDIA: Figure 8. *Video editor: Highlight the tallest bar for "coffee-grounds 1%".*

5.7.2. LAB MEDIA: Figure 8. *Video editor: Highlight all three bars for "spruce-wood*" .

5.7.3. LAB MEDIA: Figure 8. *Video editor: Emphasize downward trend in bars for "hops" and "cigarette-butt" for 5% to 10%*.

Pronunciation Guide:

1. **Biochar**
   Pronunciation link: https://www.merriam-webster.com/dictionary/biochar Merriam-Webster
   IPA: /ˈbaɪ.oʊ.tʃɑr/
   Phonetic: bye-oh-char

2. **Dehydrogenase**
   Pronunciation link: https://www.merriam-webster.com/dictionary/dehydrogenase Merriam-Webster
   (If no direct page exists, one can check specialized biochemical dictionaries.)
   IPA: /ˌdi.haɪˈdrɒ.dʒəˌneɪz/
   Phonetic: dee-high-DRO-juh-naze

3. **Triphenyltetrazolium**
   Pronunciation link: No confirmed link found
   IPA: /ˌtraɪˌfɛnɪlˌtɛtrəˈzoʊliəm/
   Phonetic: try-fen-il-tet-ra-ZO-lee-um

4. **Formazan**
   Pronunciation link: No confirmed link found
   IPA: /fɔrˈmɑ.zæn/
   Phonetic: for-MA-zan

5. **Incubation**
   Pronunciation link: https://www.merriam-webster.com/dictionary/incubation Merriam-Webster
   IPA: /ˌɪn.kjuˈbeɪ.ʃən/
   Phonetic: in-kyoo-BAY-shun

6. **Inhibition**
   Pronunciation link: https://www.merriam-webster.com/dictionary/inhibition Merriam-Webster
   IPA: /ˌɪn.hɪˈbɪʃ.ən/
   Phonetic: in-hi-BISH-un

7. **Enchytraeus albidus**
   Pronunciation link: https://www.howtopronounce.com/enchytraeus-albidus howtopronounce.com
   IPA (approx): /ɛnˈkaɪtriəs ælˈbɪdəs/
   Phonetic: en-KY-tree-us al-BID-us

8. **Sinapis alba**
   Pronunciation link: No confirmed link found
   IPA: /sɪˈnɑːpɪs ˈælbə/
   Phonetic: si-NAP-is AL-ba

9. **Analytical**
   Pronunciation link: https://www.merriam-webster.com/dictionary/analytical Merriam-Webster
   IPA: /ˌænəˈlɪtɪkəl/
   Phonetic: an-uh-LIT-ih-kul
10. **Spectrophotometer**
   Pronunciation link: https://www.merriam-webster.com/dictionary/spectrophotometer Merriam-Webster
   IPA: /ˌspɛk.trəˌfoʊˈtɒm.ɪ.tər/
   Phonetic: spek-truh-foh-TOM-i-ter
11. **Desiccator**
   Pronunciation link: https://www.merriam-webster.com/dictionary/desiccator Merriam-Webster
   IPA: /ˈdɛsɪˌkeɪtər/
   Phonetic: DES-ih-kay-ter
12. **Hydrochloric (acid)**
   Pronunciation link: https://www.merriam-webster.com/dictionary/hydrochloric Merriam-Webster
   IPA: /ˌhaɪdrəˈklɔrɪk/
   Phonetic: hy-droh-KLOR-ik