CHAPTER 17

PROXIMATE LIGNIN AND CELLULOSE

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1. INTRODUCTION

Lignin and cellulose are structural constituents of vascular plants that can make up a substantial part of litter dry mass (Table 17.1). Both compounds confer toughness to plant tissues (i.e., compressive and tensile strength; Chapter 18). Consequently, plant litter rich in these compounds tends to be highly refractory, with high concentrations particularly of lignin being conducive to slow litter decomposition (Gessner & Chauvet 1994, Berg & McClaugherty 2003).

On leaf litter decaying in streams, both biomass accumulation and sporulation activity of fungi decrease as litter lignin concentrations increase, suggesting that the negative effect of lignin is mediated at least partly through an impact on fungal decomposers (Gessner & Chauvet 1994, Maharning & Bärlocher 1996). In addition, lignin and cellulose concentrations may influence litter palatability to leaf-shredding invertebrates and hence litter consumption by these shredders. Freshwater invertebrates typically lack the enzymatic complements to digest cellulose and lignin; therefore, diets rich in these compounds are of poor nutritional quality to shredders, and this may have negative consequences for their survival, growth rate and fecundity (Bärlocher 1985, Suberkropp 1992, Graça 1993, Rong et al. 1995). However, some taxa (e.g. some *Tipula* species) may gain access to at least cellulose by means of a symbiotic cellulose-degrading gut flora (Kukor & Martin 1987, Martin 1987).

A variety of methods have been used to determine cellulose and lignin in plant tissues (e.g., McLellan et al. 1991, Van Soest et al. 1991, Hatfield et al. 1999). One simple approach, which has been widely used for forage fibre analyses and litter decomposition studies in both terrestrial and aquatic environments, consists of determining the residual weight of samples following successive removal of various tissue constituents. The first step is the extraction of components soluble in an acid detergent. Results by Ryan et al. (1990) suggest that with tree leaves and wood this approach produces similar results as the somewhat more complicated 'forest

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products analyses'. Since the approach does not necessarily determine concentrations of cellulose and lignin as defined chemically, the fractions resulting from the forage fibre method are referred to as proximate cellulose and lignin.

The aim of the method presented here is to assess the concentrations of proximate lignin and cellulose in plant litter. Concentrations are determined gravimetrically using the acid-detergent fibre procedures proposed by Goering & Van Soest (1970) with slight modifications.

Table 17.1. Concentrations of proximate lignin (Gessner & Chauvet 1994) and cellulose (Gessner, unpubl. data) in undecomposed leaf litter as determined with the fibre forage method by Goering & Van Soest (1970). Values are means ± 1 SD.

Leaf species	*Lignin (% leaf dry mass)*	*Cellulose (% leaf dry mass)*
Fraxinus excelsior	6.8 ± 0.3	18.6 ± 1.0
Prunus avium	8.4 ± 1.0	16.3 ± 0.3
Alnus glutinosa	8.0 ± 0.7	13.8 ± 1.5
Corylus avellana	13.3 ± 0.9	23.3 ± 1.6
Platanus hybrida	30.9 ± 0.8	24.8 ± 1.0
Fagus sylvatica	25.5 ± 0.8	32.2 ± 2.8
Quercus ilex	18.5 ± 1.0	27.8 ± 3.0

2. EQUIPMENT, CHEMICALS AND SOLUTIONS

2.1. Equipment and Material

- Analytical balance
- Desiccator
- Dried sample powder ground to pass a 0.5-mm mesh-screen
- Eight screw-cap extraction tubes (approx. 40 ml, pressure-resistant)
- Dry bath or water bath (100 °C) with submersible rack holding at least 8 tubes
- Sixteen crucibles, Gooch type, porosity no. 2
- Filter manifold or individual units adapted for holding 8 crucibles (individual pressure regulation preferable)
- Pump for creating vacuum in filtration systems
- Hot plate or kettle for boiling H_2O
- Eight small trays (e.g. 10 × 15 cm) resistant to 72% sulphuric acid
- Latex gloves
- Eight acid-resistant spatulas or glass rods (about 8 cm long)
- Drying oven set at 105 °C
- Muffle furnace set at 550 °C

2.2. Chemicals

- Sulphuric acid, 0.5 M (reagent grade)
- Hexadecyltrimethylammonium bromide = Cetyltrimethylammonium bromide (CTAB), 20 g l^{-1}
- Decahydronaphtalene (reagent grade)
- Acetone (reagent grade) in spray bottles
- Sulphuric acid, 72% by weight (reagent grade)

2.3. Solutions

- Solution 1: Acid detergent solution: prepare 0.5 M sulphuric acid from low-molarity stock solution, check molarity by titration, adjust if necessary, then add the detergent CTAB (20 g l^{-1}) and stir. During handling of acid wear laboratory coat, security glasses and latex gloves.
- Solution 2: Prepare sulphuric acid at 72% by weight as described below. Weigh required amount of water into a volumetric flask and add the calculated amount of H_2SO_4 in *small* portions and *very slowly* with occasional swirling. Caution: heat production with risk of explosion hazard! Constantly cool flask in a water bath (e.g. sink). Allow sufficient time for cooling. Do not fill up flask to calibration mark. Finally let cool to 20 °C and adjust to exact volume. At all times during handling of acid wear laboratory coat, security glasses and latex gloves.
- Preparation of an acid solution: Given an acid at a concentration of A% and a density, δ, an acid at the concentration of X% is obtained as follows:
  - In mass units (for 100 g of acid solution):
    100 · (X/A) of acid at the concentration A%
    100—100 · (X/A) of H_2O
  - In volumetric units (e.g. in ml):
    100 · (X/A)/D of acid at the concentration A%
    100—100 · (X/A) of H_2O

  For example, for sulphuric acid at 72% starting with 96% (δ = 1.83 g cm^{-3}):
  - For 100 g of solution:
    100 · (72/96) = 75.0 g of acid at 96%
    100—75.0 = 25.0 g of H_2O
  - Or in volume units:
    100 · (72/96)/1.83 = 41.0 ml of acid at 96%
    100—100 · (72/96) = 25.0 ml of H_2O

3. EXPERIMENTAL PROCEDURES

3.1. Sample Preparation

1. Weigh clean and oven-dry crucibles to the nearest 0.1 mg.

2. Weigh air-dry sample ground to pass a 0.2 mm-mesh screen (245—255 mg to the nearest 0.1 mg) and place in extraction tube.
3. Weigh same amount of sample in ignited, tared porcelain or aluminium pans for determining moisture content and ash-free dry mass.
4. Add to tubes 20 ml of acid-detergent solution and 0.4 ml decahydronaphatalene.

3.2. Acid-Detergent Fibre Determination

1. Heat tubes to boiling for 5—10 min in a water bath with occasional swirling.
2. Reduce heat as boiling begins to avoid foaming. Boil for 60 min from onset of boiling. Adjust boiling to a slow, even level.
3. Filter tube content on a tared Gooch crucible set on a filter manifold. Use light suction! Recover particles in tubes quantitatively. Break up the filtered mat with a spatula or glass rod and wash twice with hot water (90—100 ºC). Rinse sides of the crucible in the same manner.
4. Repeat wash with acetone until it removes no more colour. Break up all lumps so that the solvent comes into contact with all particles of fibre.
5. Suck the acid-detergent fibre free of acetone and dry overnight at 105 ºC.
6. Place oven-dry crucible in desiccator for 1 h and then weigh to nearest 0.1 mg.
7. Calculate acid-detergent fibre (ADF) as follows:

$$\frac{W_0 - W_t}{W_S} \cdot 100 = ADF \qquad (17.1)$$

where: W_0 = weight of the oven-dry crucible including fibre
W_t = tared weight of the oven-dry crucible
W_S = oven-dry sample weight.

8. Correct value for moisture content of sample.

3.3. Acid-Detergent Lignin and Cellulose Determination

9. Cover the contents of the crucible with cooled (15 ºC; water bath) 72% H_2SO_4 and stir with a spatula or glass rod to a smooth paste breaking all lumps.
10. Fill crucible about half with acid and stir. Let spatula or glass rod remain in crucible.
11. Refill with 72% H_2SO_4 and stir at hourly intervals as acid drains away. Crucibles do not need to be kept full at all times, but samples must be covered continuously. Three additions of acid suffice. Keep crucible at 20—23 ºC.
12. Filter off after 3 h as much acid as possible with vacuum (start with weak vacuum).
13. Wash contents abundantly with hot water until free from acid. Rinse and remove stirring rod.
14. Dry crucible overnight at 105 ºC.

15. Place crucible in desiccator for 1 h and weigh to the nearest 0.1 mg.
16. Ignite crucible in a muffle furnace at 550 ºC for 3 h and then cool to 105 ºC.
17. Place in desiccator for 1 h and weigh.
18. Calculate acid-detergent cellulose (ADC) as follows:

$$\frac{L_a}{W_S} \cdot 100 = ADC \qquad (17.2)$$

where: L_a = loss due to 72% H_2SO_4 treatment
W_S = oven-dry sample weight.

19. Calculate acid-detergent lignin as follows:

$$\frac{L_i}{W_S} \cdot 100 = ADL \qquad (17.3)$$

where: L_i = loss upon ignition after 72% H_2SO_4 treatment
W_S = oven-dry sample weight.

20. Correct values for moisture content of sample.

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