# Leaching Effects on Lignin Phenol Composition of Plant Materials

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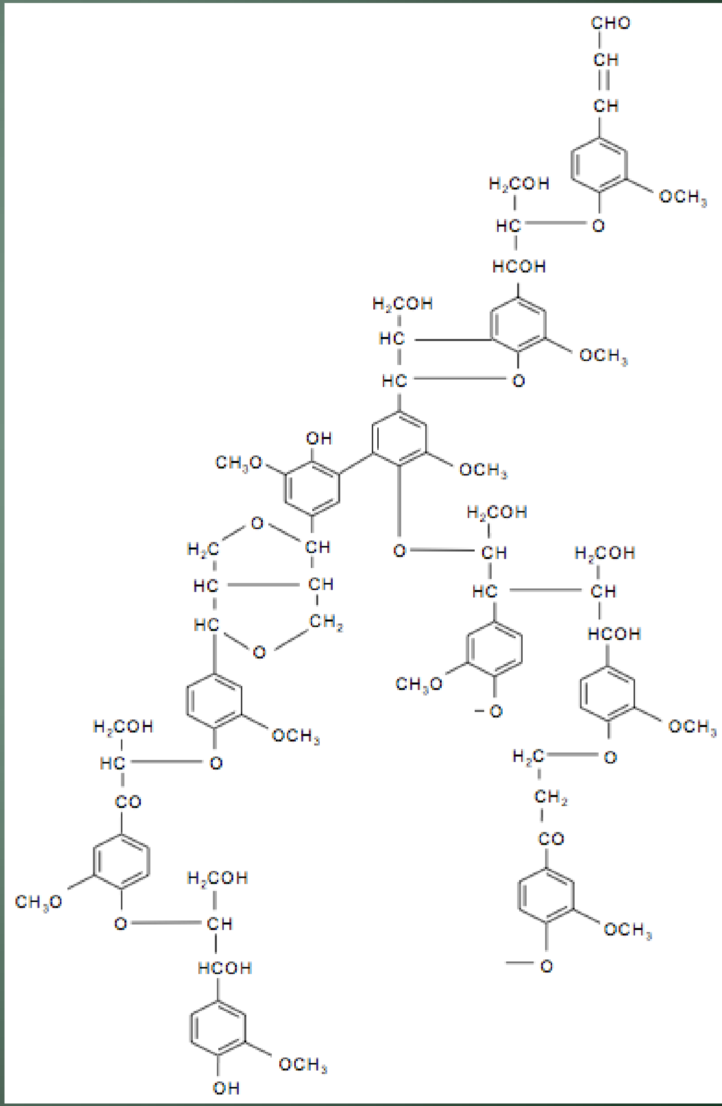
# Research Objectives

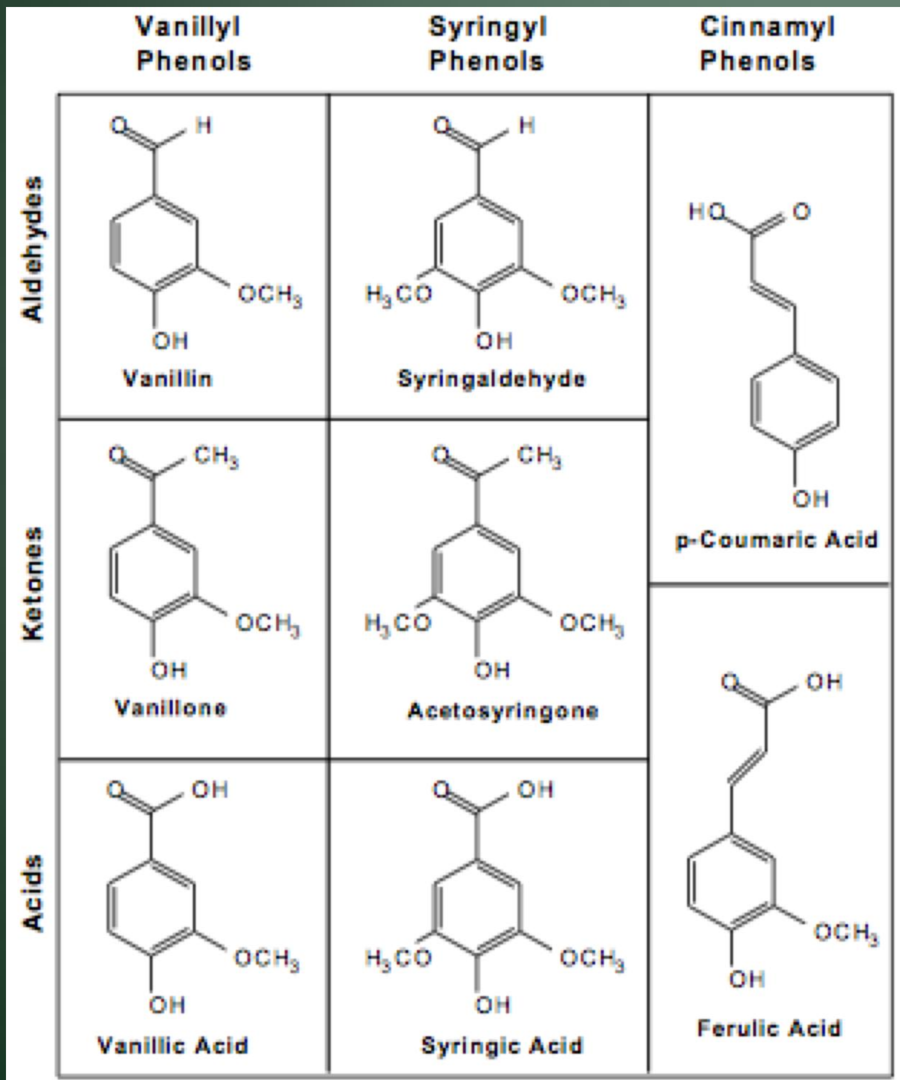
- Investigate the effects of leaching on the lignin phenol composition of various plant materials.
- Make comparisons between the leachate and ground plant material and between plant species classifications.
- Investigate relationships between sample processing and lignin phenol compositions.

# Background

Lignin is a ubiquitous, complex chemical compound that is derived from wood and as such, can be used as a tracer for terrestrially derived organic carbon in aqueous systems.



# Background, cont.




Eight lignin phenol monomers make up the structure of lignin polyphenols.
These vanillyl, syringyl, and cinnamyl phenols derive from the tissues of terrestrial plants.
Due to their structure and functional groups, lignin phenols differ in their reactivity.

# Background, cont.

- Previous studies have demonstrated that leaching can affect lignin phenol composition and can confound interpretation of lignin phenol results.
- This project will investigate leaching on various plant and tissue types. Subsamples will be ground and leaching conducted on whole and ground samples. Leachates and ground parent materials will be analyzed for lignin phenol composition.

The following table lists the abbreviations for plant species, tissue type, and classification used for the research project

| Plant Species | Tissue Type | Classification |
|---|---|---|
| SR = Sequoia Redwood | W = wood | A = woody angiosperm |
| CO = Canyon Live Oak | B = bark | a = non-woody angiosperm |
| TP = Torrey Pine | L = leaves | G = woody gymnosperm |
| FP = California Fan Palm | R = roots | g = non-woody gymnosperm |
| | N = needles | |

- On the following graphs, each color represents a plant species.
- Square: ground material leachate; circle: whole material leachate; triangle: ground parent material.

# Experimental Methods

- Sample Collection
- Sample Preparation
- Lignin Analysis and Data Collection

# Sample Collection

Various types of senescent plant materials (bark, wood, roots, leaves, needles) representing angiosperm (flowering, enclosed seed bearing) and gymnosperm (exposed seed bearing) classifications were collected from the ground around the location of the desired plant species.

# Sample Collection cont.

The following table lists the species and plant materials collected under their classification.

| Angiosperm | | Gymnosperm |
|---|---|---|
| Monocot | Dicot | |
| California Fan Palm – Bark (FP-B) | Canyon Live Oak – Wood (CO-W) | Sequoia Redwood – Wood (SR-W) |
| | | Sequoia Redwood – Root (SR-R) |
| | | Sequoia Redwood – Bark (SR-B) |
| | | Sequoia Redwood – Leaves (SR-L) |
| | | Torrey Pine – Needles (TP-N) |
| | | |
| | | |

# Sample Collection cont.

California Fan Palm
*Washingtonia Filifera*

Sequoia Redwood
*Sequoia Sempervirens*

# Sample Collection cont.

Canyon Live Oak
*Quercus Chrysolepis*

Torrey Pine
*Pinus Torreyana*

# Sample Preparation

- Samples were rinsed with nanopure water and dried at 50°C.
- Approximately half of the material was ground.
- The whole and ground materials were leached in nanopure for 24 hours in the dark at 4°C.
- The leached samples were filtered, and the filtrate (aka the leachate) was collected and frozen for lignin phenol analysis.

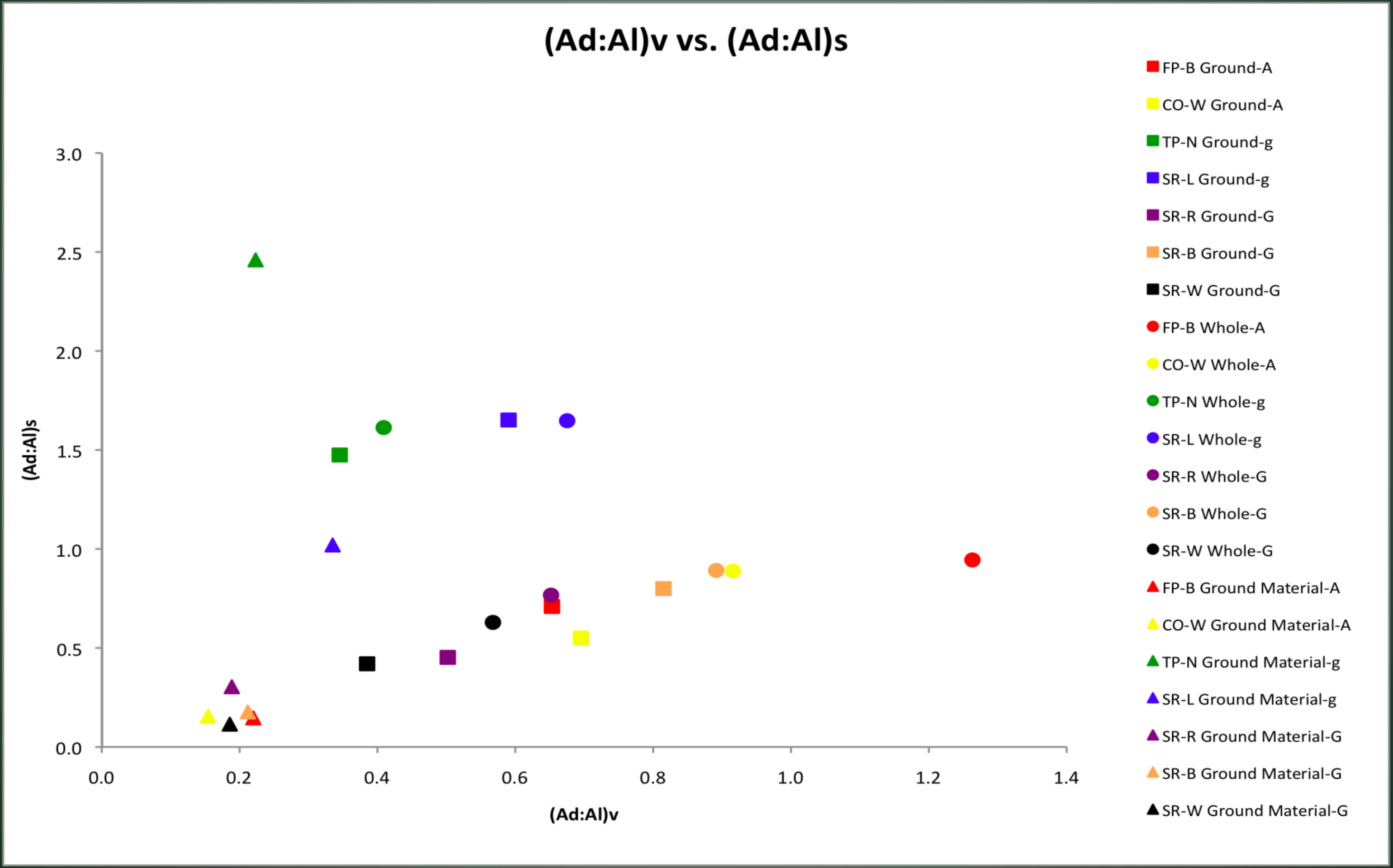
# Lignin Analysis and Data Collection

- Lignin is too large to analyze intact so it must be broken down into smaller lignin phenols.
- Subsamples of the leachates and ground parent materials were oxidized with cupric oxide and heated to 150°C for several hours, thereby cleaving ester, ether, and C-C linkages.
- Lignin phenols were then protonated using sulfuric acid followed by extraction with ethyl acetate.
- Molecular level analysis on individual lignin phenols was performed using gas chromatography-mass spectrometry.

# Lignin Analysis and Data Collection cont.

Example gas chromatography-mass spectrometer used for the analysis.

# Results

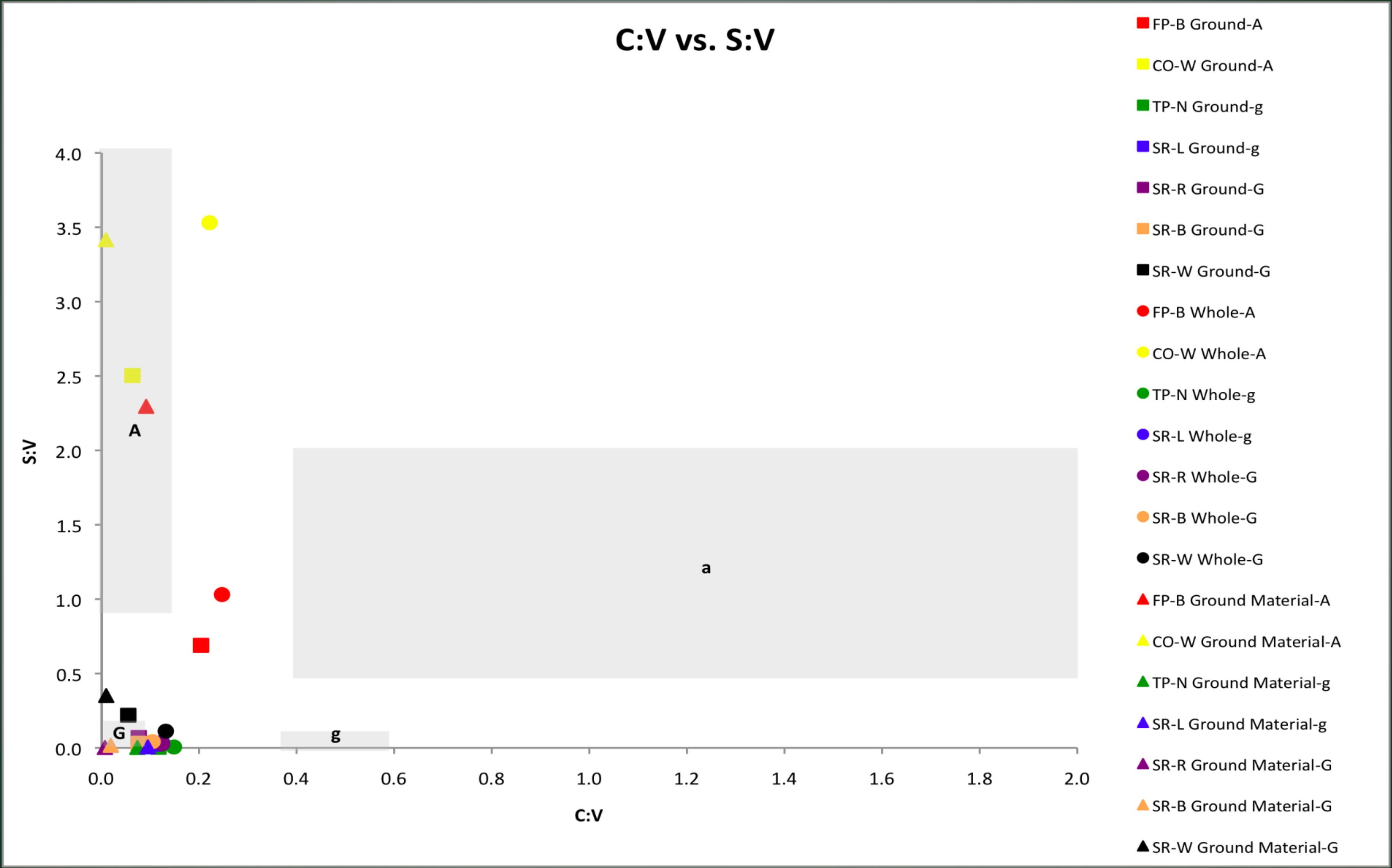


(Ad:Al)v vs. (Ad:Al)s
(Ad:Al)s
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
(Ad:Al)v
FP-B Ground-A
CO-W Ground-A
TP-N Ground-g
SR-L Ground-g
SR-R Ground-G
SR-B Ground-G
SR-W Ground-G
FP-B Whole-A
CO-W Whole-A
TP-N Whole-g
SR-L Whole-g
SR-R Whole-G
SR-B Whole-G
SR-W Whole-G
FP-B Ground Material-A
CO-W Ground Material-A
TP-N Ground Material-g
SR-L Ground Material-g
SR-R Ground Material-G
SR-B Ground Material-G
SR-W Ground Material-G

# Results cont.

- There was a general trend of elevated Ad:Al ratios in the leachates relative to the ground parent material, particularly in the whole material leachate.
- The TP-N leachates were the only samples in which the leachates had lower $(Ad{:}Al)_s$ values than the ground parent materials.
- Differential fractionation in woody vs. nonwoody tissues.

# Results cont.



# Results cont.

- The gray boxes represent traditionally defined parameters for woody and non-woody angiosperm and gymnosperm classifications.
- Some samples from this experiment fell outside traditionally defined parameters.

# Discussion

- Elevated Ad:Al ratios have typically been interpreted as indicators of degradation or decomposition of organic matter due to preferential degradation of aldehydes relative to acids.
- However, elevated Ad:Al values have also been shown to occur in leachates due to lignin fractionation, in which acids are more soluable and thuse, are more readily leached than aldehydes and ketones.
- The elevated Ad:Al values observed for the leachates illustrate the effects of lignin fractionation on the observed lignin phenol compositions and demonstrate the complexity of interpreting data.

# Discussion cont.

- Ratios of syringyl phenols (unique to angiosperms) to ubiquitous vanillyl phenols and of cinnamyl phenols (found in non-woody tissues) to vanillyl phenols have been used to distinguish between angiosperms and gymnosperms, and between woody and non-woody plant tissues.
- The fact that samples fell outside of traditionally defined parameters illustrates the difficulty of accurate interpretation of the ratios and the possible need to expand these ratios.

# Conclusions

- Effects such as lignin fractionation due to leaching can drastically alter the lignin phenol signatures in natural samples and will therefore affect interpretation of data, as demonstrated in comparisons between ground materials and their corresponding leachates.
- There was an effect of grinding on lignin composition as differences in lignin phenols were also observed between the leachates from the whole and ground materials.

Physical processes such as grinding and biogeochemical processes such as leaching can alter lignin compositions. In natural systems, plant materials are broken down, leached, permeate soils and sorb and desorb onto mineral surfaces and are also subject to photo- and microbial degradation and these processes will affect lignin phenol composition in samples. Therefore, understanding and knowledge of these processes and their impact will have important implications for accurate interpretion of lignin phenol data.