AN ABSTRACT OF THE THESIS OF

Yvan M. Alleau for the degree of Master of Science in Oceanography presented on July 26, 2002.

Title: Characterization of Organic Matter Quality and Content in Columbia River Suspended Particulate Matter by Cupric Oxide Oxidation

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Abstract approved: _____

Fredrick G. Prahl

The organic content of the Columbia River suspended particulate matter (SPM) results from the input of autochthonous (phytoplankton) and allochthonous (terrestrial vascular plants) production. The contribution of these two sources appears seasonal and responds to factors such as rainfall, runoff, river flow, light and nutrients availability. While numerous studies have focused on the phytoplankton input to this system, very little is known about the nature of the land-derived contribution.

One possible biomarker for terrestrial organic matter (OM) is lignin, a compound solely found in vascular plant cell walls. Upon alkaline CuO oxidation, lignin yields a series of eight major vanillyl (V), syringyl (S) and cinnamyl (C) phenols. Their relative composition provides insights about the origin (i.e., plant type) and level of degradation of samples, through the use of a property-property (i.e., S/V - C/V) plot and the vanillic acid /vanillin [$(Ad/Al)_v$] index, respectively.

Analysis of mostly bulk SPM samples collected on glass fiber filters (GFC) and some size-fractionated SPM samples from the Columbia River and its estuary, yielded a detailed evaluation of the strengths and weaknesses of the CuO oxidation technique as well as new biogeochemical insights to the origin and fate of the

terrestrial OM at the land-sea interface. A thorough study of the CuO oxidation products from these samples revealed that GFC filters were not suitable for lignin analysis. Use of such media resulted in lower compound recovery and an artificial increase of the degradation index $(Ad/Al)_v$. Experiments showed that this artifact was mainly due to the composition of the GFC filters $[Si(OH)_4]$ and at some level was sensitive to the particulate organic matter quality.

However, not all the parameters resulting from the CuO oxidation of the Columbia River samples were compromised by the GFC problem. Yields of three n-fatty acids (non-lignin compounds) from the CuO oxidation treatment of samples showed good correlation with compositional parameters such as $(C/N)_{at}$ and chlorophyll-a concentration and provided tracers of autochthonous OM. Compared to other rivers in the continental US and to the Fraser River (Canada), OM in the Columbia River appeared enriched in particulate nitrogen (PN) due to its high phytoplankton contribution. Results for land-derived CuO products showed that most of the allochthonous OM in the Columbia River originated yearlong from gymnosperm wood with an increase in contribution from angiosperm non-woody tissue evident during spring freshets. As a whole, no differences were seen between samples from the river and estuary possibly owing to the overall sampling strategy employed in this study. The CuO oxidation method holds promise as an analytical way to simultaneously visualize terrestrial vascular plants and phytoplankton contribution to OM.

Characterization of Organic Matter Quality and Content in Columbia River
Suspended Particulate Matter by Cupric Oxide Oxidation

by

Yvan Alleau

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Yvan M. Alleau, Author

ACKNOWLEDGMENTS

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DEDICATION

A mes parents, Maria et Michel

A mon frère, Georges

A la mémoire de mon grand-père, Biquet

CHARACTERIZATION OF ORGANIC MATTER QUALITY AND CONTENT IN COLUMBIA RIVER SUSPENDED PARTICULATE MATTER BY CUPRIC OXIDE OXIDATIOIN

1: INTRODUCTION

Soil humus and land-derived vascular plant tissues together represent 77% of the earth's active organic carbon (OC) reservoir (Hedges, 1992) and account for 50% of the global primary production (Martin et al., 1987; Hedges, 1992). Therefore, terrestrial organic matter (OM) potentially has an important impact on the global carbon cycle. A major feature of this cycle (Figure 1.1) is the extreme efficiency of OC recycling. A close look at the land-sea interface portion of the C-cycle shows that only 0.1% (0.1×10^{15} gC/yr) of the global net primary production (110×10^{15} gC/yr) is ultimately buried in marine sediments (Hedges, 1992), principally (80%) in near-shore environments (Gearing et al., 1977; Hedges and Mann, 1979; Hedges, 1992). It appears that more than 75% of the OC delivered by rivers to oceans (0.4×10^{15} gC/yr, approximately 0.5% of the terrestrial primary production) is not preserved in marine sediments. The small size of OC storage reservoirs on land, the high marine-derived OC contribution (POC rain of 7×10^{15} gC/yr at 100 m), and the low burial rate of OC in marine sediments, imply a very efficient recycling of the OM and especially terrestrial OM (99% recycled) somewhere very near the land-sea interface (Ittekkot, 1988; Hedges, 1992; Keil et al., 1997).

Compositional examination of OM in nearshore sediments confirms that autochthonous production is the dominant contributor (Emerson and Hedges, 1988). This is astonishing given that one might expect the refractory land-derived OM to be well-preserved in marine sediments compared to the more labile marine-derived OM. It is therefore of primary importance to ascertain not only the quantity but also the

quality of OM introduced to coastal regions by rivers and the processes responsible for its diagenetic fate in the marine environment.

Terrestrial Primary Production 60 x 10^{15} gC/yr		Marine Primary Production 50 x 10^{15} gC/yr

Riverine POC Discharge 0.2 x 10^{15} gC/yr

Marine POC Rain At 100 m 7 x 10^{15} gC/yr

Marine Sediment 0.1 x 10^{15} gC/yr
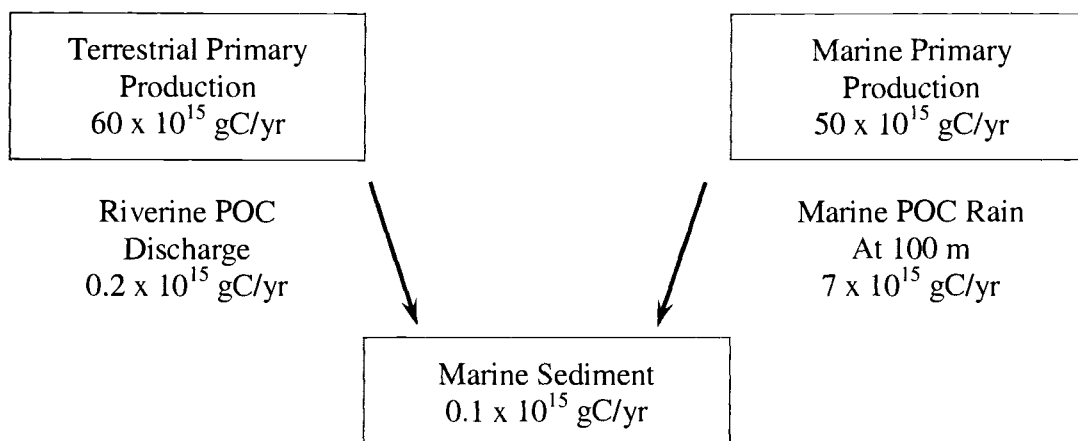
Figure 1.1 Land-sea interface portion of the Organic Carbon cycle
Data from Hedges (1992), Global biogeochemical cycles: progress and problems, Table 2 and Figure 2

Given the importance of vascular plant-derived OM as a component of the C-cycle, more studies concerning its distribution and fate at the land-sea interface appear appropriate. Scientists have previously attempted to relate specific molecules in the organic matter pool of soils to plant sources (Eglinton and Murphy, 1969). To be of value as a tracer (or biomarker), a molecule must be structurally unique and biogeochemically stable (Hedges and Mann, 1979 a and b; Reeves, 1992; Ertel and Hedges, 1985). A variety of tracers have been used as source indicators of land-derived OM in soils and sediments. These include lipid components such as n-alkanes, diterpenoid acids, n-alcohols and sterols as well as bulk compositional parameters such as C:N atomic ratios and stable isotopic (δ^{13}C) composition of total organic carbon (TOC). Nevertheless, their applications have encountered limitations due to degradation or to multiple sources of input of similar composition (Onstad et al., 2000).

A major terrestrial biomarker is lignin. This phenolic macromolecule is uniquely found in cell walls of vascular plants and aids in plant rigidity and protection against

attach by microorganisms (Sarkanen and Ludwig, 1971; Kirk, 1984; Eriksson et al., 1990). Its high resistance to degradation and its wide distribution in natural environments make it a promising geochemical tracer (Ishiwatari and Uzaki, 1987; Hedges and Ertel, 1982 b). Lignins have been used extensively to characterize vascular plant contributions in a variety of aquatic environments, including coastal waters and sediments (Hedges and Parker, 1976; Hedges and Mann, 1979 b Hedges and VanGeen, 1982; Gough et al., 1992; Steinberg et al., 1987; Gadel et al., 1990; Keil et al., 1998), rivers (Ertel and Hedges, 1984; Hedges et al., 1984,1986; Onstad et al., 2000), estuaries (Requejo et al., 1986; Prahl et al., 1997) and lakes (Hedges and Ertel, 1982 b, 1984; Ishiwatari and Uzaki, 1987).

Upon alkaline CuO oxidation (Hedges and Ertel, 1982), lignin yields a series of eight major vanillyl, syringyl and cinnamyl phenols with aldehydic, ketonic and acidic side chains (Figure 1.2). The relative proportions of these monomers allow the distinction between various broad types of vascular plant tissues (Hedges and Mann, 1979 a; Goni and Hedges, 1992) and can be related to lignin content and diagenesis in plant tissues and samples (Hedges and Mann, 1979 b). In particular, the vanillic acid / aldehyde ratio [$(Ad/Al)_v$] is used as an estimate of the degradation level of plant derived matter, as it linearly increases as decomposition proceeds (Ertel and Hedges, 1984; Ertel et al., 1984; Hedges and Ertel, 1988 c; Moran et al., 1991; Goni et al., 1993). However, recent studies (Benner et al., 1990; Goni, 1992; Goni and Hedges, 1992) have revealed that some fresh vascular plant tissues, such as non-woody angiosperms and gymnosperms, contain large amounts of vanillic acid ester-bound to polysaccharides. Although fresh, these tissues yield $(Ad/Al)_v$ ratios greater than 0.3 upon CuO oxidation. This complicates the use of this parameter as a precise diagenetic indicator as fresh tissues are typically considered to be characterized by $(Ad/Al)_v$ of 0.15 ± 0.05 (Hedges et al., 1982) and elevated values are considered indicative of diagenetic alteration.
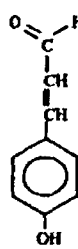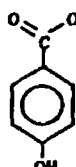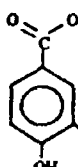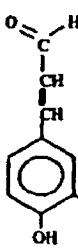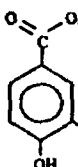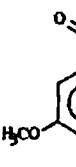
Figure 1.2 Eight major CuO oxidation products of lignin
Hedges et al. (1988b)

Being the most important pathway accounting for the presence of terrestrial matter preserved in sediments (Hedges, 1992), the transfer of OM from land to sea via rivers appears to be a key link in the global carbon cycle. It is therefore surprising that very few studies examined this transfer in any detail. The Land Margin Ecosystem Research (LMER) program was designed to investigate the complex relations between watersheds, rivers and estuaries, as well as to determine the impacts of the growing human population on coastal areas. One of the LMER programs, the Columbia River Estuary Turbidity Maximum project (CRETM), was conducted from 1990 to 2000 within the Columbia River and its estuary. Its aim was to investigate the chemical, biological and physical processes occurring in Estuary

Turbidity Maxima (ETM) of this river-dominated estuary, as well as to quantify the effects at the land-sea margin of anthropogenic changes within the watershed (Simenstad et al., 1994 b).

The history of people in the Pacific Northwest has long been linked to the Columbia River and its tributaries as potential sources of fresh water and food and as a means of transportation. Today these uses still exist but their relative importance has changed and additional uses of the river have appeared. In particular, within the last century, the river's flow has been modified by damming for irrigation and related agricultural needs as well as for hydropower and tourism. Because of the close dependency between people and rivers, numerous studies have been conducted to understand the physical, chemical, geological and biological mechanisms happening in rivers including the Columbia River, to determine and potentially control the impacts of an ever-growing human population (Prahl and Coble, 1994; Baross et al., 1994; Small and Morgan, 1994; Reed and Donovan, 1994;Simenstad et al., 1994 a and b; Prahl et al., 1997; Sullivan et al., 2001; Covert, 2001). However, rare are the studies that mention lignin results. As a consequence, very little is known about lignin content (i.e., concentration) and occurrence (i.e., seasonality) in the Columbia River and its estuary. This is surprising given that terrestrially-derived organic matter along with autochthonous production are the only two natural sources of OM to the river, which eventually discharges its load into the coastal area.

In this study, I analyzed samples collected during the LMER-CRETM project to address critical questions concerning the origin, quantity, quality, seasonality and fate of suspended OM with an emphasis on land-derived OM. Bulk and size-fractionated (> 64 μm and < 64 μm) suspended particulate matter (SPM) from two particular locations in the Columbia River, one upstream at river mile 53 (RM53) and the second within the estuary (ETM, North and South channels) were analyzed by CuO oxidation (Section 2.1.1). Results from both locations were also compared to assess downstream changes. These data were also compared to results from samples collected in July 1999 in the free-flowing Fraser River (Canada) in order to test if the

features observed in the Columbia River were unique to the drainage of this river system or also expressed in another major Pacific Northwest river system. This work is the first detailed, lignin-based study of suspended particulate organic matter in the Columbia River.

During this study, my efforts also aimed to understand and improve the CuO oxidation technique. These efforts eventually yielded a detailed evaluation of the strengths and weaknesses of this technique. Specifically, this thesis provides the first report of problems resulting from the use of glass fiber filters (GFC) to collect samples for lignin analysis by the CuO technique. Results show that such filters yield low sample recovery and artificially increase the degradation index $(Ad/Al)_v$.

2: METHODS

2.1 SAMPLING

2.1.1 Sample Collection

Samples examined in this study were collected during the Columbia River Estuary
Turbidity Maximum (1990-2000) project as part of the Land-Margin Ecosystem
Research program, funded by the National Science Foundation (NSF). Sites of
sample collection in the Columbia and Fraser rivers are identified in Figures 2.1 a)
and b) (coordinates in Appendix I) and an overview of the watershed for each river is
provided in the following sections.

In situ measurements and sample collections were done aboard the *RV Robert
Gordon Sproul* for all cruises but one done aboard the *RV Wecoma* in February 1998.
Shipboard equipment included a conductivity-temperature-depth profiler (CTD)
fitted with an optical backscatter (OBS) sensor. Water samples were collected using a
high volume vertical-profiling water pump delivering ~ 510 L/min (at the vessel's
deck) and allowing measurement of suspended particulate matter concentration and
composition (elemental carbon and nitrogen, chlorophyll, CuO oxidation products).
Different materials and methods of collection, filtration and storage were used
depending upon the technique to be used for sample analysis and are addressed in
Section 2.2.

2.1.2 The Columbia River Watershed

The Columbia River is the largest Pacific Northwest River and is the fourth biggest
river in the United States in terms of discharge (Van der Leeden et al., 1990) with a
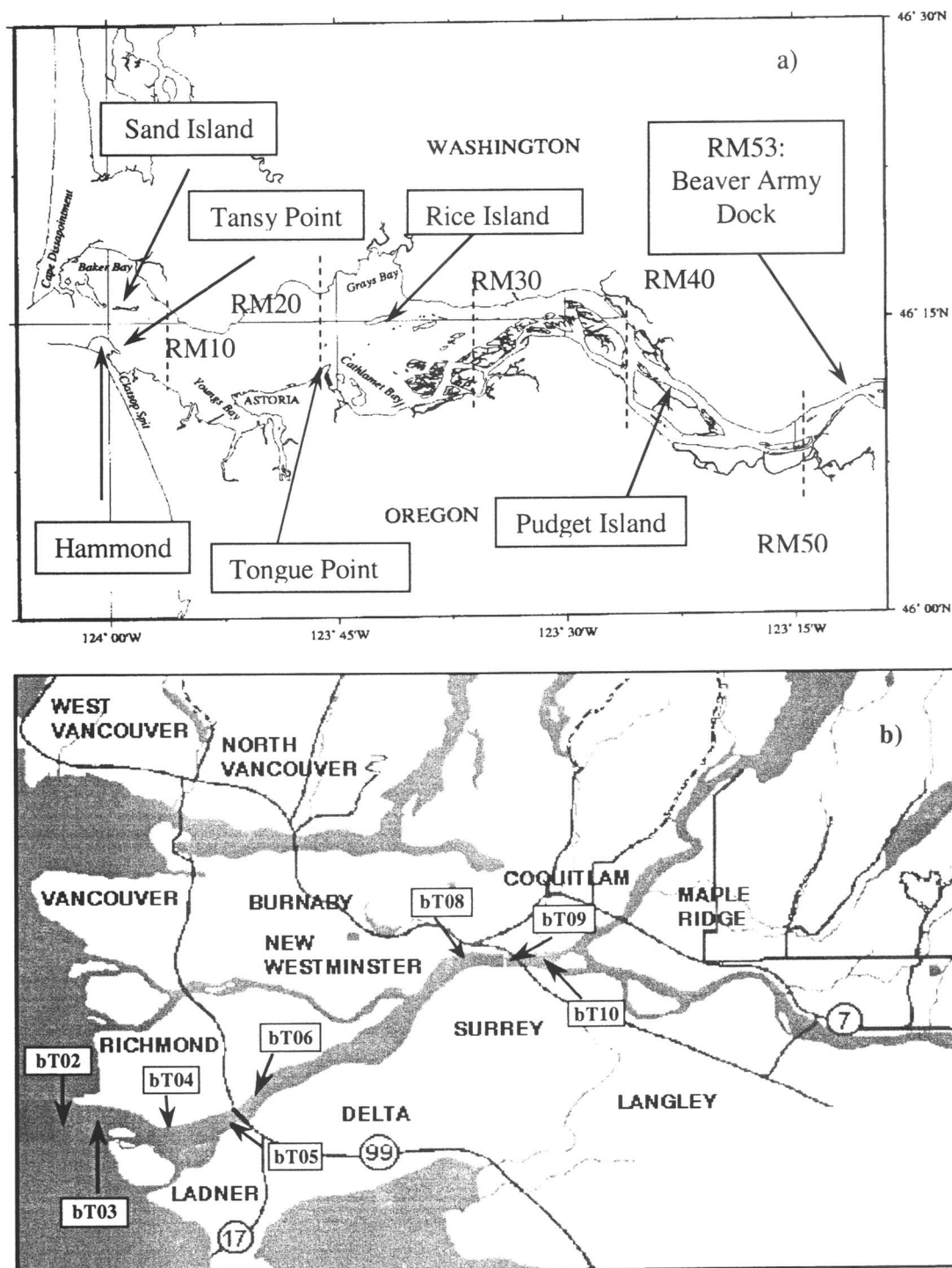
Figure 2.1 Sampling sites
(a) Columbia River: Map outline P. Covert. RM: river mile, (b) Fraser River: bT:
transect bottom sample. Map: http://depts.washington.edu/cretmweb/CRETM.html.

mean annual flow of 7,500 m^{3}s^{-1} (Berner & Berner, 1996). Along with its tributaries, it drains a 667,000 km^2 watershed (Meybeck, 1979; Sherwood et al., 1990), and delivers about 10^7 metric tons of sediment per year to its estuary (Simenstad et al., 1990). Its drainage basin encompasses parts of seven American states and one Canadian province. The Columbia River main stem is 1,950 km long (1,200 km in the US) and drops from an elevation of about 800 m at its origin in British Columbia to about 2.5 m above sea level at Bonneville dam (RM145). It flows through four mountain ranges: the Rockies, Selkirks, Cascades and the Coast Range.

The two principal tributaries of the Columbia River are the Snake and the Willamette Rivers, both flowing in Oregon. The Snake River alone accounts for 49% (327,000 km^2) of the Columbia River basin and has an annual average flow of 1,500 m^{3}s^{-1}. The Willamette River only slightly exceeds 4% (29,500 km^2) of the Columbia River basin. It flows 497 km before reaching the Columbia River at Portland and has an average discharge of 900 m^{3}s^{-1} (Fuhrer et al., 1996). Eventually, the Columbia River discharges into the Pacific Ocean through its estuary at Astoria creating a plume extending into the Pacific Ocean. The plume responds to seasonal wind and current pattern and typically extends southward and offshore during summer (upwelling period) and northward and alongshore during the winter (downwelling period). The Columbia River is the main source of fresh water to the northeastern Pacific Ocean with a contribution ranging from 60% in winter and 90% in summer (Barnes et al., 1972). This massive fresh water intrusion into the ocean carries along sediments, which contribute at least 95% of the sedimentary material being deposited on the southern Washington continental shelf (Hedges et al., 1984b).

The climate and the vegetation within the watershed changes as the Cascade Mountains divide it in very distinct eastern and western subbasins. This natural barrier between the cold inland air masses and the warmer maritime air masses creates two very different hydrological and climatic areas. The eastern portion displays a sub-humid to arid, more continental climate and accounts for 92% of the total watershed area but only contributes 76% of the water discharge principally

through runoff from snowmelt (April to July). The vegetation is composed of sparse coniferous forests, snowbrush, sagebrush and large steppe zones. Large zones are heavily exploited for cropland and pasture. In contrast, the western subbasin displays a wetter climate, contributes 8% to the total area but accounts for 24% of the water discharge (Fox et al., 1984; Orem, 1968; Good and Jay, 1978) especially during the winter months. Forests are remarkably different from those east of the Cascades. Coniferous species dominate everywhere particularly on the mountain flanks. Few deciduous patches are found in the coastal area.

2.1.3 The Fraser River Watershed

The Fraser River is the fourth biggest river in Canada in terms of discharge with a mean annual flow of 2,720 m^3s^{-1}. The river originates in the Rocky Mountains, in Mount Robson Provincial Park near the Alberta border. Its main tributaries are the Nechako, the Chilcotin and the Thompson Rivers. The river flows between the Cascade Range and the Coast Mountains before it empties through three main estuarine channels into the Strait of Georgia at Vancouver. Along with its tributaries, the Fraser River drains a 232,300 km^2 watershed and free-flows 1,370 km through the province of British Columbia.

In contrast to the Columbia River, whose flow pattern has been greatly modified by damming since the late 60's, the Fraser River's hydrograph was not changed much in the 20th century. The flow amplitude changes on a year to year basis as the river is not controlled by dams or levees but the general seasonal pattern remained unchanged, displaying four hydrological periods: (1) winter, lowest flow in the year (< 1,000 m^3s^{-1}); (2) spring, flow increases due to the melting of the snow pack from the Ranges (1,500-6,700 m^3s^{-1}); (3) early summer the flow stays high and starts decreasing after mid-summer to a low of 1,700 m^3s^{-1} in early fall; (4) fall, discharge decreases to winter values.

Coniferous forests cover the majority of the Fraser drainage basin, although a mixture of deciduous forests and grasses covers the eastern mountainous part. In its valley part, deciduous forests and cropland mainly surround the river.

2.2 TECHNIQUES OF ANALYSIS

2.2.1 SPM Concentration

Large volumes of water were collected into 20 L carboys using the submerged pump. Surface (1m below surface), middle and bottom (1m above bottom) water were collected at most stations. After homogenization, sub-samples, typically 1-3 L, were pressure-filtered (N_2, 100 kPa) through a 1.0 μm pre-weighed polycarbonate membrane filter (90 mm dia., Poretics). The filters were then folded into quarters, placed into clean Petri dishes and oven-dried (60°C, 24 hrs). SPM concentration (mg/L) for fresh water samples was determined from the difference in weight between the loaded and unloaded filter, divided by the volume filtered. Size-fractionated SPM samples were collected as described in paragraph 2.2.3.

2.2.2 POC/PN Concentration

Bulk SPM samples were obtained by vacuum filtering 200 to 500 mL of water onto pre-combusted (450°C, 4hrs) glass fiber filters (25 mm dia. GF/F, Whatman). These were then oven-dried (60°C, 24hrs) and stored in clean Petri dishes until analysis. Other samples analyzed included Lake Washington Standard Mud (LWSM) as well as different woodchips (Douglas fir, Red alder).

Typically, 5 to 10 mg of sample (or a filter containing the sample) were used for elemental analysis. Analysis for particulate organic carbon (POC) and nitrogen (PN)

was performed by flash combustion at 1020°C using a Carlo Erba NA 1500 (Carlo Erba Instruments) and the instrumental setup of Verardo et al (1990). Prior to analysis, a series of standards (0.1-1 mg of acetanilide N: 10.36%, C: 71.09% or l-cystine: N: 11.66%, C: 29.99%) were run along with blanks to calibrate the analyzer response. Comparison of earlier results from analysis of acid-treated and untreated samples from the Columbia River (Prahl et al., 1998) indicated negligible inorganic carbon content. Therefore, our samples were not acid-treated (e.g., Hedges and Stern, 1984; Verardo et al., 1990). As a result, reported data represent total particulate carbon and nitrogen measurements. The precision of the technique used for elemental analysis was typically ± 2% for C and ± 3% for N.

2.2.3 CuO Oxidation

Samples analyzed by alkaline CuO oxidation included bulk and size-fractionated SPM. Bulk samples were collected by pressure filtering (N_2, 100 kPa) 3-8 liters of water onto pre-combusted (500°C, 24 hrs) coarse glass fiber filters (GFC, 90 mm diameter, 1.2 μm nominal pore size, Osmonics®). Placed into plastic Petri dishes, they were then oven-dried (60°C, 24 hrs) and stored until analysis. Size-fractionated samples (> 64 μm and < 64 μm) were obtained from large volumes of water (60 to 80 L) pumped into 24 L high-density polyethylene carboys. The water was pre-filtered immediately onboard the ship using a 64 μm mesh sieve to separate coarse from fine particles. The > 64 μm fraction was then washed with DI water into a scintillation vial and stored frozen. A Millipore® tangential flow filtration system (Hernandez and Stallard, 1998) loaded with a Pellican® millipore cassette (0.45 μm polycarbonate membrane) was used onboard ship to concentrate the pre-filtered volume containing the fine fraction (< 64 μm) down to approximately one liter. This volume was further concentrated by centrifugation and stored frozen in pre-cleaned

glass bottles. Back at Oregon State University, all ultrafiltration samples were freeze-dried, weighed, homogenized by grinding in a ball-mill and stored in scintillation vials until needed for analysis.

Because much of the organic matter in sedimentary materials exists as macromolecules (e.g., lignins, polysaccharides, proteins, phospholipids), it does not lend itself to direct analysis by a standard gas chromatographic–flame ionization (GC-FID) technique. It must first be chemically degraded into smaller molecules that lend themselves to such analysis. Among the various techniques developed for the measurement of lignin, the most commonly used is CuO oxidation (Hedges and Ertel, 1982). This technique was extensively used in this study to investigate the lignin and other macromolecular compound content and composition of SPM.

Typically, lignin yields a series of phenolic compounds upon alkaline CuO oxidation among which eight monomers (Figure 1.2) are of special interest for this study. These major compounds include vanillyl (V), syringyl (S) and cinnamyl (C) phenols (Hedges and Ertel, 1982). V and S are measured in their aldehyde, ketone and acid forms, whereas C are only in acid forms. Primarily designed for lignin study, the CuO technique also yields monomeric and dimeric products from decomposition of phospholipids, proteins and carbohydrates, resulting in a whole series of other monomers and dimers (Goni, 1992; Goni and Hedges, 1992). Examples of typical CuO reaction product chromatograms are given in Figure 2.2 and include results for a standard solution (Std16, Figure 2.2 a) and for a sample from the Columbia River estuary (99aL21177B > 64 μm, Figure 2.2 b).

2.2.4 Details of the CuO Analytical Procedure

The CuO oxidation procedure used in this study was essentially that developed and described by Hedges and Ertel (1982) and further modified by Requejo (1986) and Goni (1990 b and c; 1992). Depending on the OM content of the sample, 100 to 125
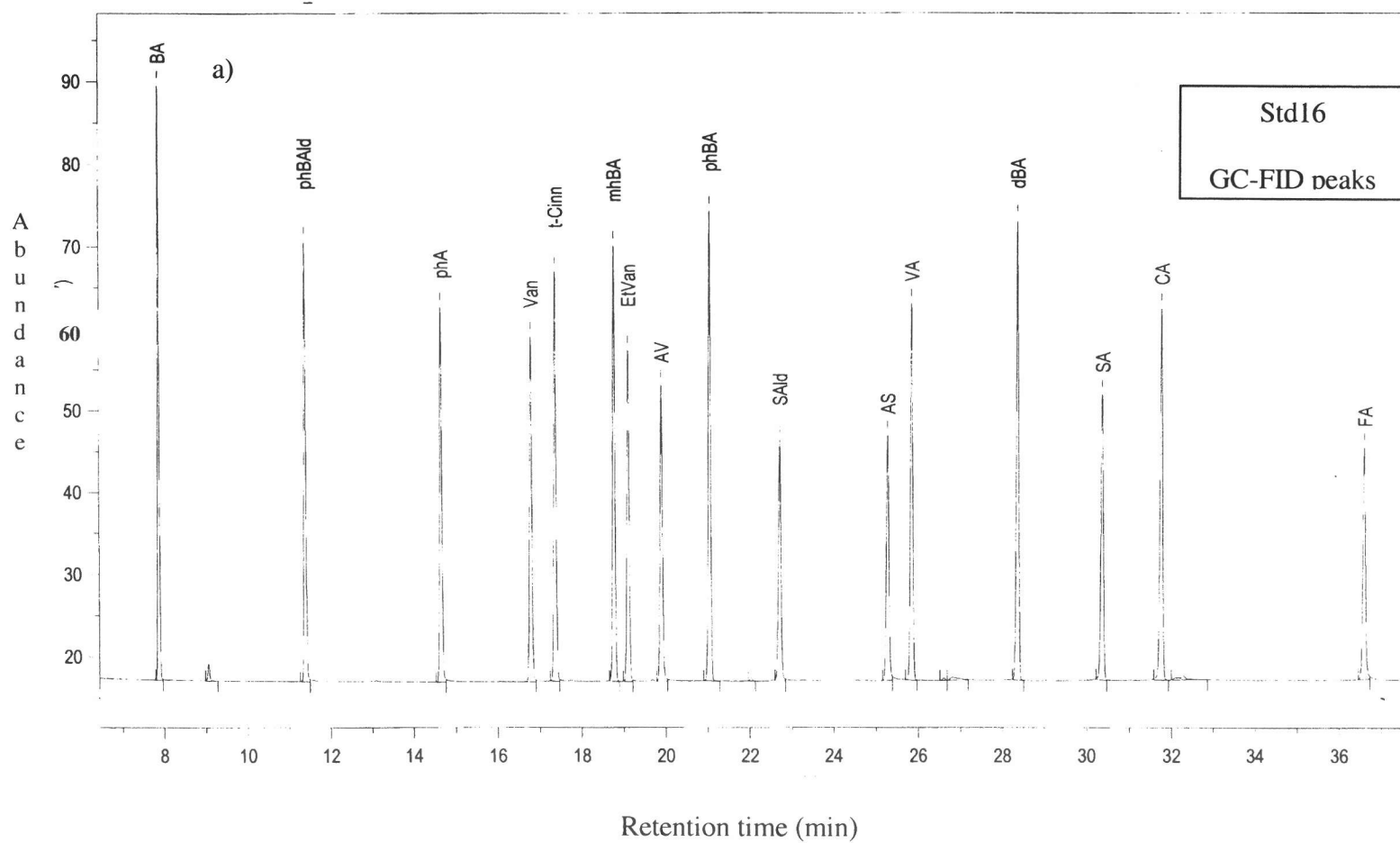
Figure 2.2 Examples of CuO reaction product chromatograms
a) Standard 16 (Std16), b) Columbia River estuary sample 99aL21177B > 64 um. Retention times differ slightly between a) and b) depending upon the GC settings
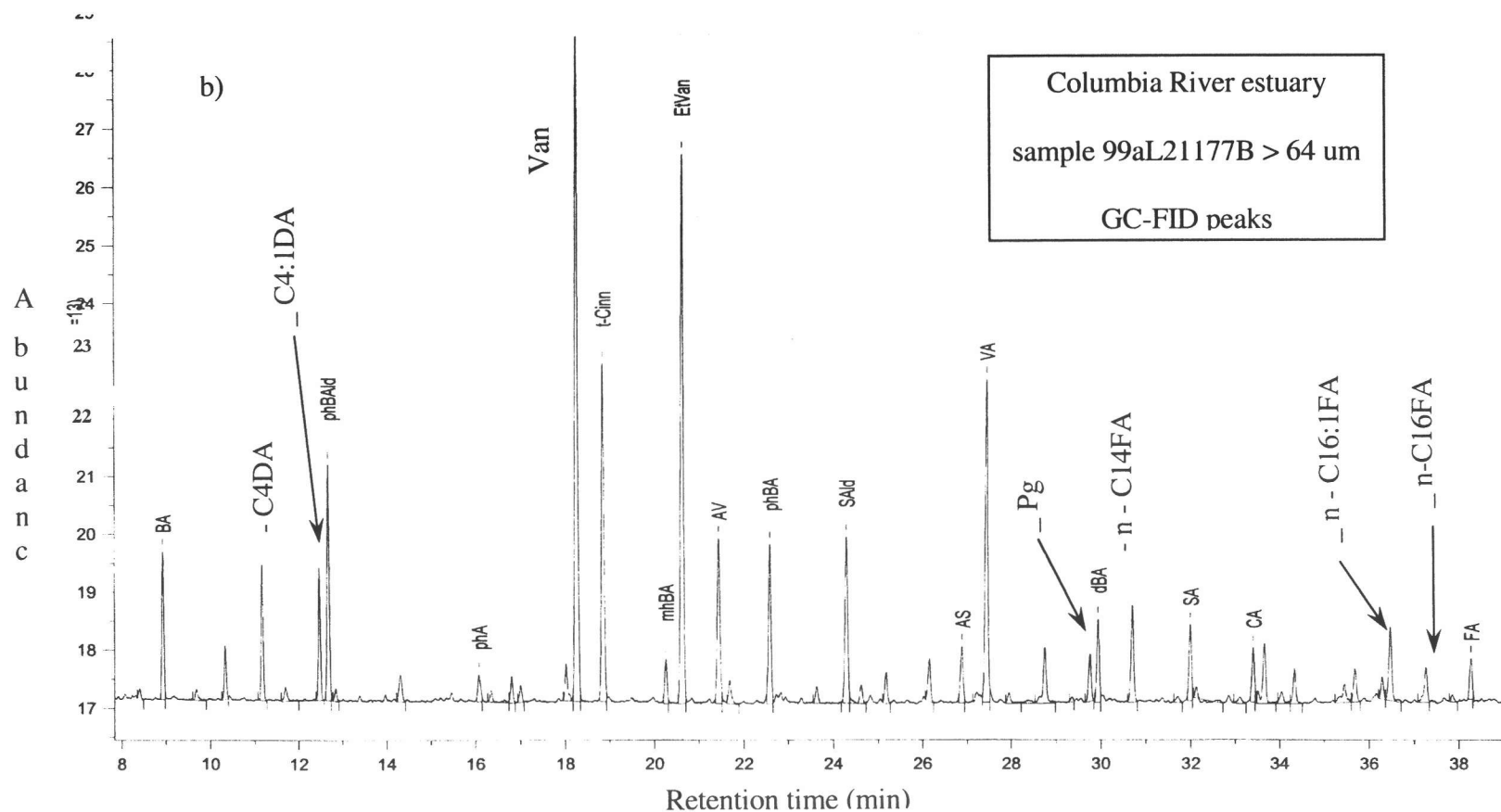
Figure 2.2 Continued. BA: benzoic acid, C4DA: butane-1,4-dioic acid, C4:1DA: 2-butene-1,4-dioic acid, phBald: p-hydroxybenzaldehyde, phA: p-hydroxyacetophenone, Van: vanillin, t-cin: t-cinnamic acid, mhBA: m-hydroxybenzoic acid, Et-Van: ethylvanillin, AV: acetovanillone, phBA: p-hydroxybenzoic acid, SAld: syringaldehyde, AS: acetosyringone, VA: vanillic acid, Pg: p-hydroxyphenylglyoxylic acid, dBA: 3,5 dihydroxybenzoic acid, n-C14FA: tetradecanoic acid, SA: syringic acid, CA: t-p-coumaric acid, n-C16:1FA: hexadecenoic acid, n-C16FA: hexadecanoic acid, FA: t-ferrulic acid

mg of material were oxidized with 1 g of CuO (CH_2Cl_2-extracted) under alkaline conditions (7 mL of 8% by weight NaOH, previously purged for 1 hour with N_2), along with 100 mg of $Fe(NH_4)_2(SO_4)_2,6H_2O$ (O_2 scavenger). Unlike Hedges and Ertel (1982), no radio-labeled tracer was added to the minibombs (prior to the bombing) as a recovery standard. The reaction was carried out at 155°C for 3 hours in four 10 mL stainless steel Monel minibombs loaded into a 200 mL Parr bomb. The latter was secured and heated in an aluminum cylinder wrapped with electrical heating tape and fiberglass. The whole system was fixed on a platform shaker to insure a good mixing of the reactants. Reaction temperature (achieved in 15 min) was controlled by a Cole-Parmer DYNA SENSE temperature controller (Model 2157) fitted with an armored platinum temperature probe, later replaced by a Cole-Parmer RTD proportional temperature controller (accuracy ± 0.2°C) fitted with a 100 Ω platinum RTD probe. Unlike Goni et al. (1992), only the outside temperature was monitored. Following oxidation, 30 to 50 μl of ethyl vanillin (Et-Van, 1 mg/mL) was added to the minibombs reaction mixture as a recovery standard (Requejo et al., 1986). Samples were then acidified to pH 1 (HCl 6N) and extracted three times with ethyl ether (10 mL). The volume of the combined extracts was reduced to approximately 1 mL by roto-evaporation. At that stage, the concentrate was dried by passage through a Pasteur pipette filled with granular, anhydrous sodium sulfate (Na_2SO_4) into a 1-dram vial. The solutions were then evaporated to dryness under a gentle stream of pre-purified N_2 and either used directly for gas chromatographic analysis (see next paragraph) or stored frozen for later analysis.

2.2.5 Gas Chromatography

Sample preparation for gas chromatography (GC-FID) analysis was a variation of that described by Hedges and Ertel (1982). The frozen vials containing the dry organic residues from the alkaline CuO oxidation procedure were slowly warmed up to room temperature as were two other vials: one containing a solution of trans-

cinnamic acid (t-Cin), the other BSTFA [N, O-bis [trimethylsilyl] trifluoroacetamide with 1% TMCS (trimethylchlorosilane), Pierce Chemical]. t-Cin was used as an internal GC-FID standard, whereas BSTFA with 1 % TMCS was used as a derivatizing agent to convert the CuO oxidation products to more volatile trimethyl silyl (TMSi) ether and ester forms for subsequent GC-FID analysis. The samples were then dissolved with 1 part by volume t-cin in pyridine and BSTFA. These relative proportions were always the same to insure complete derivatization of the CuO oxidation products. The total dilution volume depended on the sample size and was typically 100 μL. Upon dilution and gentle mixing, the vial was heated at 60°C for 20 minutes in a block heater (Multi-Block H2025-1A) to insure complete derivatization. Once cooled to room temperature, an aliquot (1–2 μL) was withdrawn using a gas-tight syringe (5 μL, Hamilton Co.) and injected into the gas chromatograph for compositional analysis. It is important to note that the samples were derivatized just prior to GC-FID analysis since their composition in solution was subject to changes with time. Pyridine and the lignin phenols are light sensitive and degrade via oxidation and repolymerization. Furthermore, the TMSi derivatives are prone to breakdown by hydrolysis. These potential complications would act to compromise the analysis.

GC-FID analysis was carried out using a Hewlett Packard 5890A gas chromatograph, fitted with a 30 m x 0.25 mm i.d. DB-5 fused silica capillary column (0.25 μm film thickness, J&W Scientific), later replaced by a 30 m x 0.25 mm i.d. DB-1 fused silica capillary column (0.25 μm film thickness, J&W Scientific) to improve mixture resolution (in particular VA and AS, Figure 2.2). The samples were injected using a split ratio of ~3/50 and H_2 (~7 psi column head pressure) rather than He as the carrier gas. Separation was accomplished using temperature programming with no initial delay from 100°C to 270°C at 3°C/min and held isothermal for 10 minutes. Temperature was then increased to 300°C at 10°C/min and held isothermal for 10 minutes (total run time: 80 min). Both the injector and the flame ionization detector (FID) were maintained isothermally at 300°C throughout the analysis. Data

were collected and processed using a Chrom Perfect (v.3.5) software program (Justice Innovation, Chromatography Data Systems). Each compound was identified by comparing retention times (RT) in the sample with those found in previously run standards (Std16). The n-fatty acids were identified based on gas chromatography-Mass Spectrometry (GC-MS) analysis. Typical chromatograms are displayed in Figure 2.2 a) and b).

2.2.6 Gas Chromatography-Mass Spectrometry

The sample preparation for GC-MS analysis was the same as previously described for the GC-FID. The electron impact spectra were obtained for the TMS (tetramethylsilane) ester and ether derivatives of the CuO reaction products using a Hewlett Packard 5890 Series II Gas Chromatograph fitted with a Hewlett Packard 5971 Series Mass Selective Detector. Settings for GC-MS were the same as for GC-FID, but with helium as carrier gas. The spectra were produced at an ionization energy of 70 eV and interpreted using a HP MS Chem Station (v.c.00.07) software program. The GC-MS was used for peak identification of the CuO oxidation mixture and was applied to lignin and non-lignin compounds.

2.2.7 Concentration Calculation

An internal standard method was used to determine the concentrations for individual CuO oxidation products. The general mechanics of this method are as follows. Prior to any sample analysis, the GC-FID was calibrated using a standard composed of sixteen known compounds (Std16). Given the quantity injected (amount x) and the resultant chromatographic area (area x), a response factor (RFx) was determined for each compound in Std16 using Equation 1:

$$RF_X = \frac{(amount_X)_{Std16}}{(area_X)_{Std16}} \tag{1}$$

A response factor for each compound relative to t-cin, the internal GC-FID standard, was then calculated using Equation 2:

$$RRF_X = \frac{(RF_X)_{Std16}}{(RF_{t-cin})_{Std16}} \tag{2}$$

Once the GC-FID was calibrated and working properly, samples were analyzed. GC data obtained for samples containing the GC-FID internal standard (ISTD) t-cin and the recovery standard Et-Van were then used to assess the concentration of individual CuO oxidation products. The assessment was made from external RRF data defined through analysis of Std16 and internal data from sample analysis using Equation 3:

$$[X]_{uncor} = \frac{RRF_X \times (RF_{t-cin})_{Sple} \times Dvol \times (area_X)_{Sple}}{(weight)_{Sple}} \tag{3}$$

with:

* $Dvol$: dilution volume resulting from the introduction of t-cin prior to analysis,

* $(area_X)_{Sple}$: peak area for compound x in the sample,

* $(weight)_{Sple}$: weight of sample analyzed.

Assuming Et-Van is a valid proxy for recovery, concentrations were corrected for loss of sample that may have occurred post-bombing, during the wet chemical workup procedure. The calculation was made using Equation 4:

$$[X]_{corr} = \frac{\left(\dfrac{RRF_X}{RRF_{Et-Van}}\right) \times \left(\dfrac{(area_X)_{Sple}}{(area_{Et-Van})_{Sple}}\right) \times (amount_{Et-Van})_{Rec} \times (volume_{Et-Van})_{Rec}}{(weight)_{Sple}} \quad (4)$$

where: $(amount_{Et-Van})_{Rec}$ and $(volume_{Et-Van})_{Rec}$ were the concentration and volume of the recovery standard introduced in each sample post-bombing, respectively.

2.3 REPRODUCIBILITY OF ANALYTICAL TECHNIQUES

Prior to any sample analysis, the reproducibility of the different analytical techniques was investigated. In particular, the GC-FID precision was evaluated based on multiple reruns (Std16, samples), while the CuO technique was evaluated both by comparing results with earlier data (qualitative approach) and by carrying aliquots of the same sample through the entire oxidation-extraction process and comparing the results (quantitative approach).

2.3.1 Gas Chromatography Analysis

Several tests were done to assess the extent of analytical variability that is attributable just to GC-FID performance. They included: 1) selection of the GC-FID method (of best precision) to be used by comparing the precision of the internal (ISTDM) and external (ESTDM) method of evaluating compound concentration (Section 2.3.1.1); 2) evaluation of the selected (by test #1) ISTDM by monitoring RRF results and comparing them with previous results from this study as well as from earlier research. The precision of RRF measurements was also investigated through replicate standard analysis; and finally 3) sample composition reproducibility of duplicate injections of the same extract (Section 2.3.1.3).

2.3.1.1 Calibration lines

A standard solution (Std4) of four compounds (t-Cin, Et-Van, BA and FA as defined in Figure 2.2) was prepared. Different dilutions of this standard (Appendix II) were analyzed in replicates on the GC-FID. Plots in Figures 2.3 a), b) and c) display "amount injected vs. area measured" results obtained by using an external standard method (ESTDM) whereas plots d), e) and f) display "amount injected vs. corrected amount measured" results obtained by using an internal standard method (ISTDM). In both cases, results behaved linearly but the internal method appeared superior to the external method in several ways: 1) ISTDM correlation coefficients were higher (0.991 to 0.999) than those from ESTDM (0.911-0.941), 2) ISTDM calibration lines displayed ~ zero intercept, and 3) results from sample reruns (four times) displayed lower (i.e. better) percentage standard deviation (%Stdev: 0.85-3.52%) than for the ESTDM (10-12%). As a result the ISTDM was selected for this study.

2.3.1.2 Relative Response Factor

The GC-FID performance was continuously monitored by keeping track of the relative response factors (RRF) of each compound in Std16. This was achieved by analyzing Std16 each day (prior to any sample analysis) and by comparing the RRF with earlier results. Such data for SAld, Van, CA and FA phenols (as defined in Figure 2.2) are displayed in Figures 2.4. The RRF remained very stable throughout the study with a coefficient of variation (c. var) of 3% or better (Table 2.1, Rep[a]), indicative of a good reproducibility of the GC-FID. Higher variability (Rep[a] ~ 3.5%) was observed for C phenols (CA and FA) possibly due to the fact that these compounds were the last to elute from the column and usually displayed broader peaks. In the mean time, reproducibility based upon duplicate analysis of Std16 (Table 2.1, Rep[b]) was very good as c. var averaged 0.6%, most of the compounds

External standard method

a) Et-Van uncorrected

$y = 8E\text{-}06x + 0.0117$
$R^2 = 0.9407$

b) BA uncorrected

$y = 6E\text{-}06x + 0.056$
$R^2 = 0.9207$

c) FA uncorrected

$y = 7E\text{-}06x + 0.0186$
$R^2 = 0.9111$
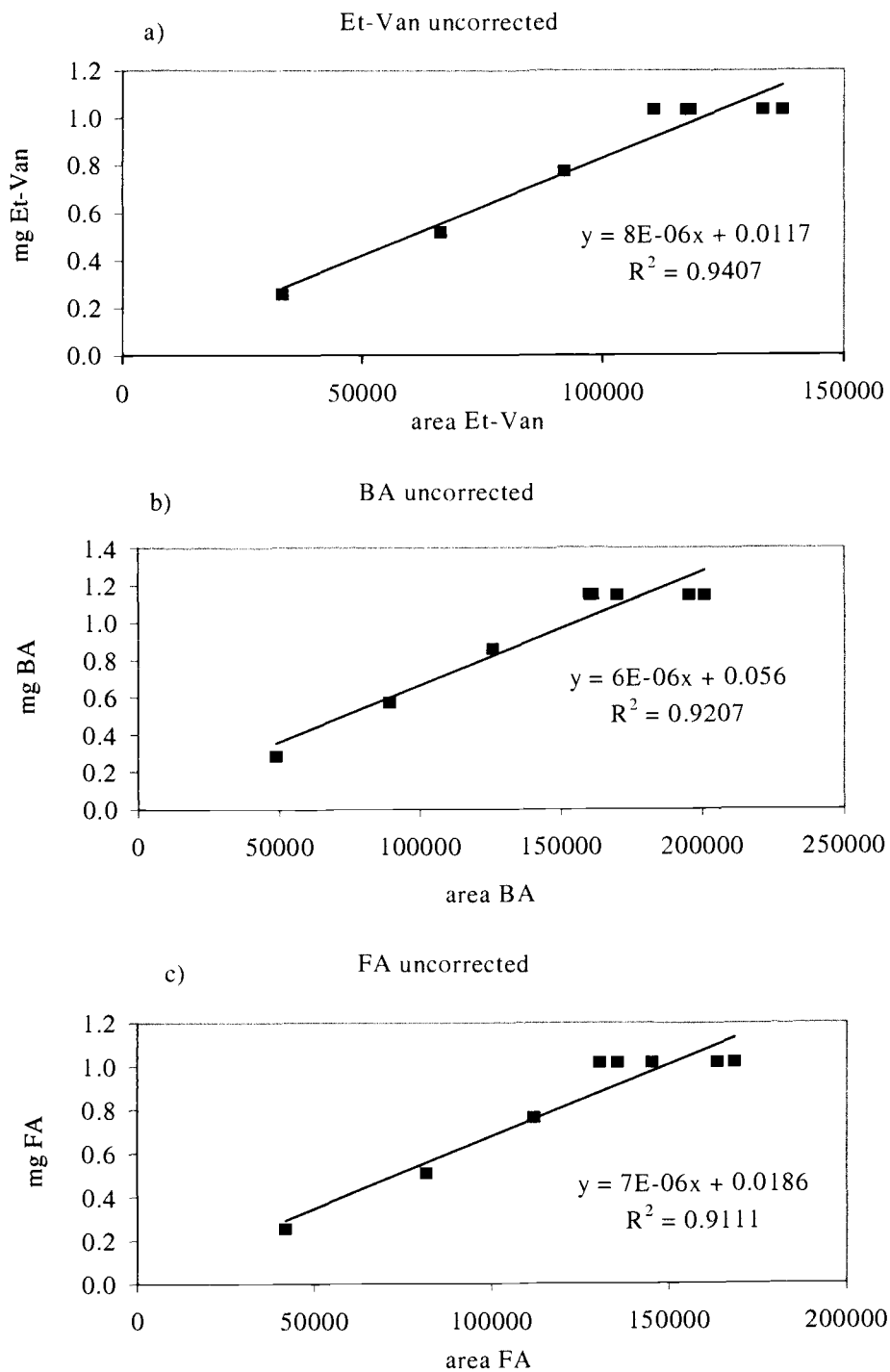
Figure 2.3 Gas chromatography calibration lines
Plots a), b) and c) refer to an external standard method. In ordinate is the injected
amount and in abscissa the measured area

d) Et-Van corrected

$$y = 1.2942x - 0.0007$$
$$R^2 = 0.9991$$

e) BA corrected

$$y = 0.978x + 0.0293$$
$$R^2 = 0.9906$$

f) FA corrected

$$y = 1.0673x - 0.012$$
$$R^2 = 0.9908$$
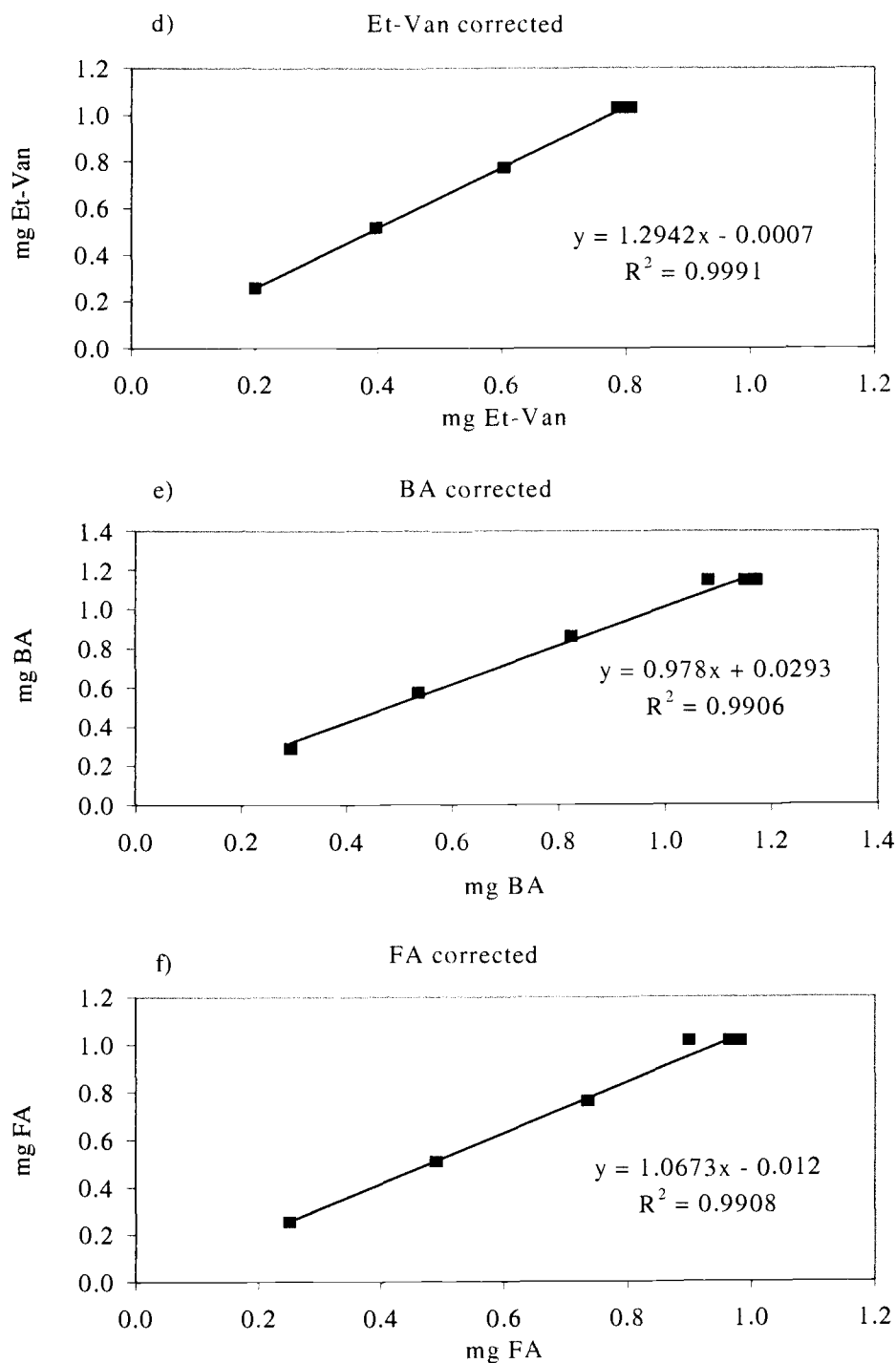
Figure 2.3 Continued

Plots d), e) and f) refer to an internal standard method. In ordinate is the injected amount and in abscissa the measured amount
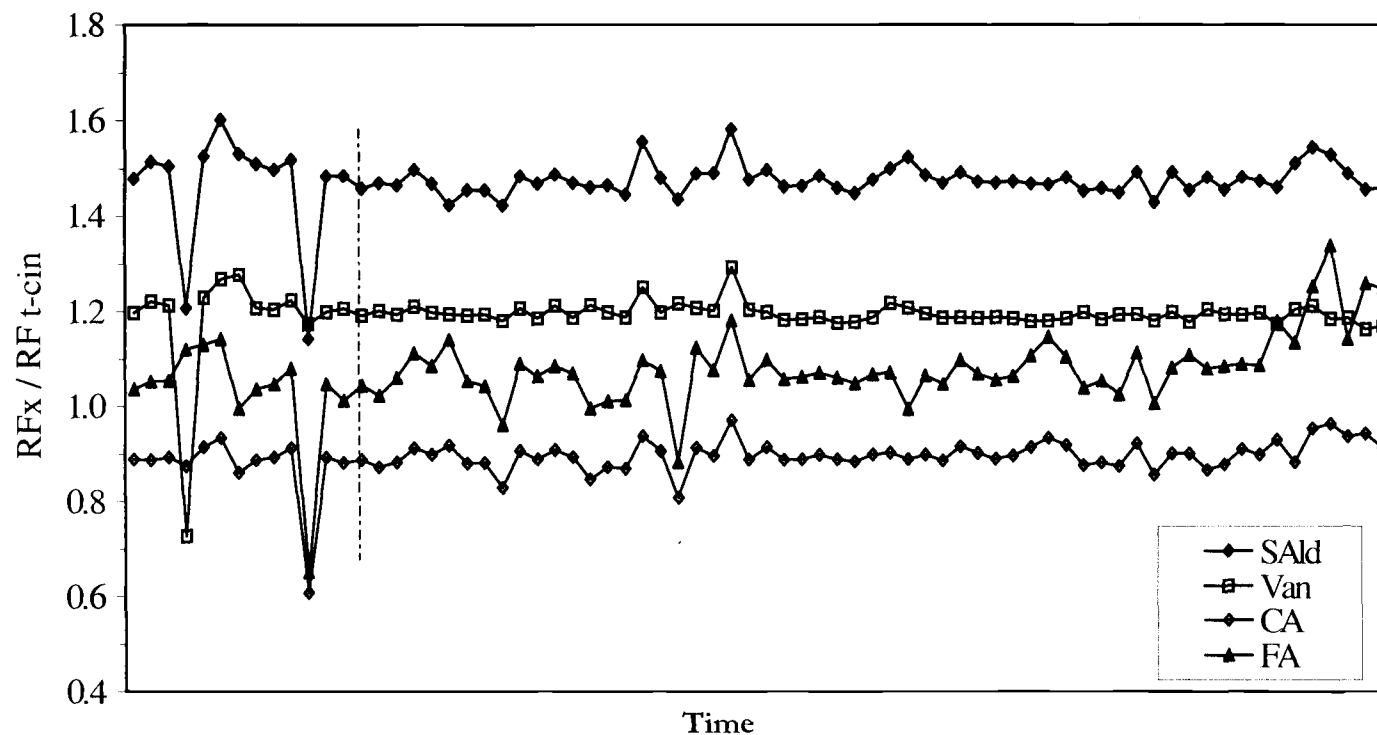
Figure 2.4 Relative Response Factor (RRF) evolution

Evolution through this study of four lignin phenols RRF (1 Syringyl, 1 Cinnamyl and 1 Vanillyl) resulting from the GC analysis of Std16. The worst case occurred with FA (c. var = 4.6%) whereas the other compounds displayed c. var < 3%. The vertical dashed line indicates the end of the preparatory work and the beginning of sample analysis. c. var : coefficient of variation

displaying results ≤ 0.4%. Here again, the highest variability was found for CA and FA (1.1% and 2.7% respectively). While the previous results showed that the GC-FID calibration was stable over the duration of my thesis, a comparison with RRF data from research conducted in our lab in 1987 and 1990 proved that it could be maintained for a longer time frame (Table 2.2). Indeed, the coefficient of variation between my results and those from 1987 and 1990 were, for each compound, comparable to those obtained solely with my data.

Table 2.1 Relative Response Factor (RRF) and reproducibility for Std16
Coefficients of variation were calculated from all the RRF data right of the dashed line in Figure 2.4. [a]: reproducibility from analysis of all Std16 solutions prepared in this work (n = 59). [b]: reproducibility based on four series of Std16 duplicates

Compound name	code	average RRF	Rep [a] %	Rep [b] %
benzoic acid	BA	1.01	2.93	0.19
p-hydroxybenzaldehyde	phBald	1.09	2.29	0.39
p-hydroxyacetophenone	phA	1.12	2.11	0.29
vanillin	Van	1.20	1.71	0.36
m-hydroxybenzoic acid	mhBA	0.93	1.38	0.36
ethylvanillin	Et-van	1.24	2.23	0.32
acetovanillone	AV	1.25	2.05	0.64
p-hydroxybenzoic acid	phBA	0.87	1.56	0.30
syringaldehyde	Sald	1.48	1.96	0.31
acetosyringone	AS	1.40	2.62	0.35
vanillic acid	VA	1.04	2.02	0.11
3,5 dihydroxybenzoic acid	dBA	0.80	2.51	0.34
syringic acid	SA	1.24	2.73	0.91
t-p-coumaric acid	CA	0.90	3.01	1.12
t-ferrulic acid	FA	1.08	4.59	2.73
		average	2.38	0.58

Table 2.2 Stability of Relative Response Factor over time
RRF for Std16 obtained in our lab over 13 years: GC calibration can be maintained
over a long period of time. /: data not available. c. var: coefficient of variation

Compound name	code	average 1987	average 1990	this study 2000	c.var %
benzoic acid	BA	1.06	1.06	1.01	2.52
p-hydroxybenzaldehyde	phBald	1.20	1.12	1.09	4.96
p-hydroxyacetophenone	phA	1.10	1.10	1.12	1.01
vanillin	Van	1.21	1.18	1.20	1.38
m-hydroxybenzoic acid	mhBA	0.90	0.92	0.93	1.50
ethylvanillin	Et-van	1.22	1.19	1.24	1.98
acetovanillone	AV	1.23	1.18	1.25	3.11
p-hydroxybenzoic acid	phBA	0.85	0.86	0.87	1.02
syringaldehyde	Sald	1.46	1.40	1.48	2.70
acetosyringone	AS	1.34	1.34	1.40	2.66
vanillic acid	VA	0.97	0.96	1.04	4.34
3,5 dihydroxybenzoic acid	dBA	0.76	0.81	0.80	3.17
syringic acid	SA	1.14	1.19	1.24	4.13
t-p-coumaric acid	CA	0.90	0.84	0.90	4.09
t-ferrulic acid	FA	1.20	1.11	1.08	5.43

2.3.1.3 Sample reruns

The two previous tests involved solely the analysis of standard solutions. They
evaluated the reproducibility of relatively "simple" compound mixtures and would
therefore account for the minimum "variability" of the technique. A necessary test of
GC-FID reproducibility involved the analysis of multiple injections of more
compositionally complex real samples (LWSM, tree woodchips and river samples).
Typical results are displayed in Table 2.3 (Columbia River estuary sample:
99aL21177B > 64 μm). As expected, reproducibility was a little lower than for
standard solutions, with c.var one order of magnitude higher (yet low) than those

observed in Table 2.1[b] for Std16 RRF. C.var were typically < 3% for S and V related
parameters and ~10% for C related parameters. The poorer reproducibility of
parameters based on C phenols observed in tests #2 and #3 might be explained by: 1)
their being the latest compounds to elute from the column and 2) their being present
in low concentration compared to other compounds.

Table 2.3 Gas chromatographic reproducibility
Reproducibility of lignin data based on multiple injection of sample 99aL21177B >
64 (June 1990, Columbia River estuary, bottom water). Compounds are defined in
Figure 2.2. c. var: coefficient of variation. Lignin in mg/g; Λ in mg/100mg OC

Sample	Van	AV	VA	SAld	AS	SA	CA	FA	V	S	C
					concentrations in mg/g						
Injection 1	0.480	0.127	0.202	0.151	0.050	0.054	0.029	0.025	0.808	0.255	0.054
Injection 2	0.482	0.124	0.198	0.147	0.048	0.051	0.024	0.022	0.804	0.246	0.046
Injection 3	0.479	0.121	0.190	0.148	0.048	0.049	0.023	0.021	0.790	0.244	0.044
average	0.480	0.124	0.196	0.149	0.049	0.051	0.025	0.023	0.801	0.248	0.048
c. var	0.3%	2.3%	3.1%	1.6%	2.6%	4.4%	13.0%	9.5%	1.2%	2.3%	11.4%

	Lignin	Λ	S/V	C/V	%Vo	%So	Vadal	Sadal	3,5dBA/V	%Reco
Injection 1	1.12	6.01	0.315	0.067	15.7	19.6	0.420	0.354	0.047	71.2
Injection 2	1.10	5.89	0.306	0.057	15.5	19.6	0.410	0.344	0.040	74.2
Injection 3	1.08	5.80	0.309	0.056	15.4	19.5	0.396	0.333	0.042	73.8
average	1.10	5.90	0.310	0.060	15.5	19.5	0.409	0.344	0.043	73.1
c. var	1.8%	1.8%	1.6%	10.4%	1.2%	0.3%	3.0%	3.0%	8.7%	2.3%

2.3.1.4 Conclusion about GC reproducibility

As a whole, the GC-FID technique displayed good reproducibility with c.var ranging
from 1 to 3% for S and V related parameters and ~10% for C parameters. The other
main outcome from these tests was that reproducibility depended upon a compound's

concentration, with lower concentrations resulting in lower reproducibility (e.g., see CA and FA, Table 2.3).

2.3.2 CuO Oxidation Technique

Three different approaches were used to assess the integrity of the CuO oxidation data for purposes of biogeochemical interpretation. The first compared results obtained from the analysis of Lake Washington Standard Mud (LWSM) with those found in the literature (qualitative approach). The second approach compared results of true replicate analysis of LWSM and Columbia River SPM (quantitative approach). Finally, the reproducibility of the technique was investigated for samples on glass fiber filter, as they constituted the majority of the samples available.

2.3.2.1 Comparison with the literature

The optimal reference material for the CuO oxidation technique is Lake Washington Standard Mud (obtained from J. Hedges, University of Washington). LWSM was prepared in 1990 by homogenizing a large mass of modern sediment collected at the bottom of Lake Washington. Aliquots of this material were analyzed throughout this study to monitor the performance of the CuO oxidation procedure. Results for the main lignin parameters as well as those from earlier studies are displayed in Figures 2.5 a), b), c) and d) (tabulated data in Table 2.4, individual phenols concentration in Appendix III). From this comparison, it appeared that my results were not different from those of Requejo et al. (1986) and Prahl et al., (1990, unpublished data), indicating that the CuO oxidation procedure was performing correctly.
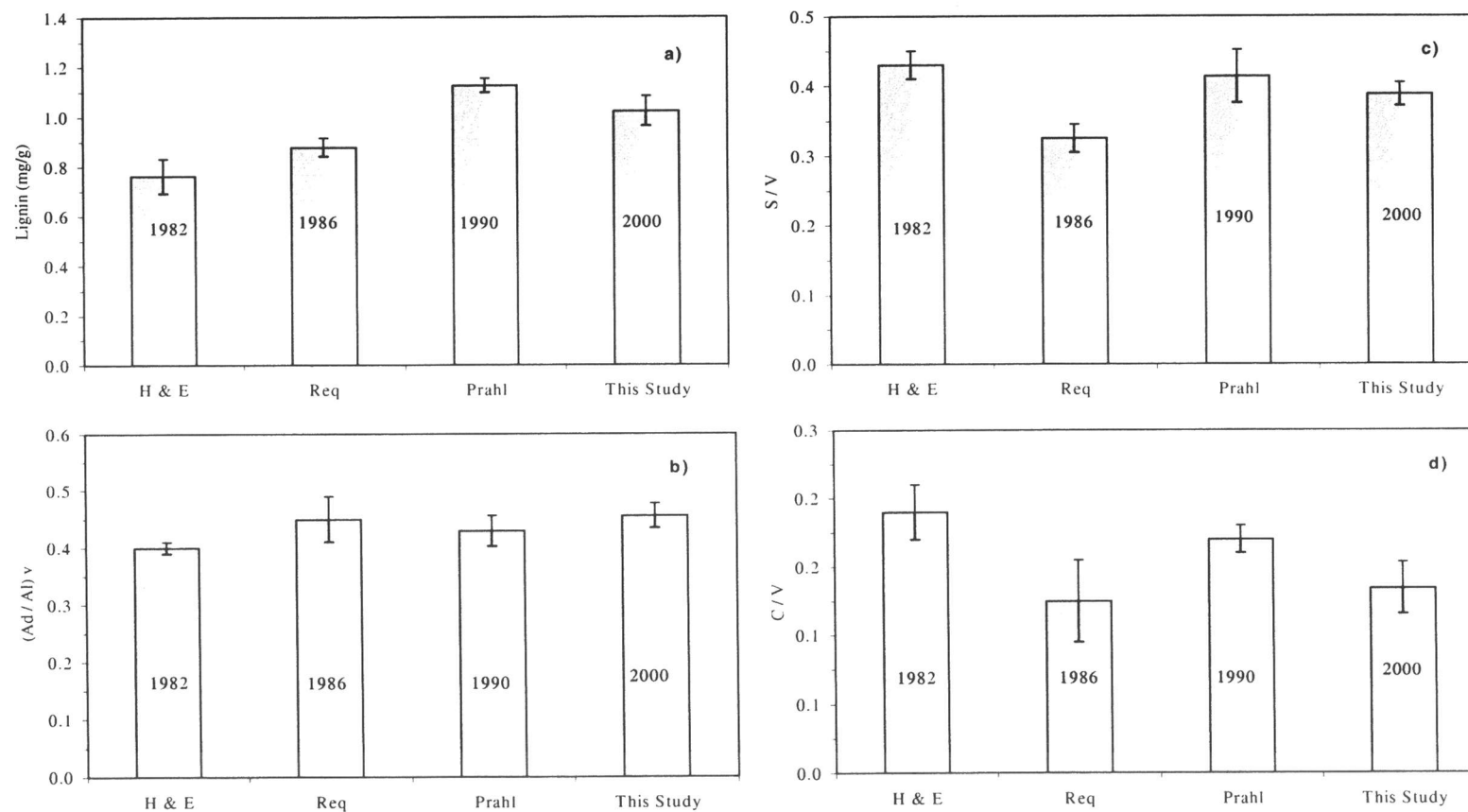
Figure 2.5 Literature comparisons of CuO oxidation products of LWSM
a) Lignin yield (mg/g), b) decomposition indicator $(Ad/Al)_v$, c) S/V and d) C/V. References are: "H&E": Hedges and Ertel (1982), n=3; "Req": Requejo et al. (1986), n=3; "Prahl": Prahl et al., unpublished data (1990), n=3; "This study": average results for LWSM samples (n=6). Error bars are the variation of measurement for multiple analyses

Table 2.4 Literature comparisons of CuO oxidation products of LWSM
Lig and Λ: lignin concentration in mg/g and mg/100mgOC, respectively. Prahl et al., (1990) are unpublished data.
/: data not available. c. var: coefficient of variation. %Reco: percentage of recovery. V, S, C concentrations in mg/g

References		V	S	C	Lig	Λ	S/V	C/V	%Vo	%So	Vadal	Sadal	%Reco
LWSM													
This study	average	0.67	0.26	0.09	1.02	2.16	0.39	0.13	19.0	21.5	0.46	0.36	64.1
%OC = 4.73%, n = 6	c. var	5.4%	7.2%	14.8%	5.9%	5.9%	4.3%	14.0%	5.6%	11.0%	4.8%	9.6%	
Requejo et al., (1986)	average	0.61	0.20	0.08	0.88	1.73	0.33	0.13	18.3	18.7	0.45	0.53	/
%OC = 5.1%, n = 3	c. var	/	/	/	4.2%	4.2%	6.2%	24.0%	/	/	8.9%	9.4%	
Prahl et al., (1990)	average	0.71	0.29	0.12	1.13	2.25	0.41	0.17	18.9	24.3	0.43	0.39	78.5
%OC = 5%, n = 3	c. var	4.8%	5.2%	0.5%	2.5%	2.5%	9.2%	5.9%	4.1%	7.3%	6.3%	16.5%	
Hedges and Ertel, (1982)	average	0.47	0.20	0.09	0.76	1.51	0.43	0.19	18.5	21.4	0.40	0.29	/
%OC = 5.05%	c. var	/	/	/	9.1%	9.1%	4.7%	10.5%	/	/	2.5%	10.3%	

2.3.2.2 Reproducibility of the method

Reproducibility of the CuO technique for both homogenized solid samples and samples on GFC filters was investigated by replicate analyses of aliquots carried through the entire procedure. Samples included solid samples of 1) LWSM (Table 2.4), 2) Columbia River estuary (99aL21177B > 64, Table 2.5) and RM53 (97cU1109M < 64, Table 2.6) as well as samples artificially put onto GFC filters including 3) LWSM, Columbia River and estuary samples (Tables A1 and A2).

Lignin results from samples (1) and (2) were comparable and assessed the high reproducibility of the technique. The c.var of most measures were ± 10%, ranging from 4 to 15% overall. Best reproducibility was obtained for V, S, S/V, %Vo, (Ad/Al)$_v$ and total lignin data (4-8%) while lower reproducibility were

Table 2.5 Reproducibility of the CuO technique
Based on results of four different "bombings" of sample 99aL21177B >64 (June 1990, Columbia River estuary, bottom water). Compounds are defined in Figure 2.2. c. var: coefficient of variation. Lignin in mg/g; Λ in mg/100mg OC

	Van	AV	VA	SAld	AS	SA	CA	FA	V	S	C
					concentrations in mg/g						
Sample 1	0.480	0.127	0.202	0.151	0.050	0.054	0.029	0.025	0.808	0.255	0.054
Sample 2	0.483	0.147	0.210	0.160	0.061	0.062	0.037	0.037	0.840	0.283	0.074
Sample 3	0.500	0.163	0.218	0.170	0.072	0.065	0.034	0.032	0.881	0.307	0.066
Sample 4	0.484	0.138	0.174	0.160	0.059	0.047	0.029	0.027	0.796	0.266	0.056
average	0.487	0.144	0.201	0.160	0.060	0.057	0.032	0.030	0.831	0.278	0.063
c. var	1.9%	10.5%	9.6%	4.8%	14.9%	14.2%	12.7%	17.2%	4.6%	8.2%	14.8%

	Lig	Λ	S/V	C/V	%Vo	%So	Vadal	Sadal	3,5dBA/V	%Reco
Sample 1	1.118	6.008	0.315	0.067	15.7	19.6	0.420	0.354	0.047	71.2
Sample 2	1.197	6.434	0.337	0.088	17.4	21.6	0.435	0.387	0.050	69.7
Sample 3	1.254	6.741	0.348	0.075	18.5	23.4	0.436	0.383	0.050	55.5
Sample 4	1.118	6.01	0.334	0.070	17.4	22.1	0.358	0.296	0.040	68.7
average	1.172	6.298	0.334	0.075	17.3	21.7	0.412	0.355	0.047	66.2
c. var	5.7%	5.7%	4.1%	12.4%	6.7%	7.3%	8.9%	11.9%	10.2%	11.0%

found for C and C/V (~13%). Such feature echoed observations made earlier in the

evaluation of the GC-FID reproducibility. Table 2.6 reveals two other major results.

The first result is that slightly lower reproducibility was obtained for the small size-

fractionated samples (< 64 μm) as compared to the large size-fractionate samples

(>64 μm). One explanation is that lignin is mainly contained in bigger particles.

Therefore, and as seen previously, lower reproducibility is achieved for compounds

of low concentration. The second result concerns the n-fatty acids (n-FA) used in this

study as tracer of autochthonous OM (see Section 2.4). Among the six compounds

considered, three (n-$C_{14, 16:1, 16}$FA) displayed reproducibility of ~12 %, similar

Table 2.6 Reproducibility of n-FA
Based on results of four different "bombings" of sample 97cU1109 M<64 (October 1997, RM53, middle water column). Compounds are defined in Figure 2.2. c. var: coefficient of variation. Lignin in mg/g; Λ in mg/100mg OC

Sample	C4DA	C4:1DA	Pg	n-C14FA	n-C16:1FA	n-C16FA	S	V	C
				concentrations in mg/g					
Sample 1	0.049	0.047	0.019	0.085	0.066	0.053	0.045	0.150	0.009
Sample 2	0.042	0.057	0.023	0.081	0.068	0.045	0.048	0.165	0.010
Sample 3	0.037	0.082	0.033	0.077	0.079	0.047	0.056	0.177	0.011
Sample 4	0.052	0.030	0.005	0.073	0.059	0.062	0.041	0.142	0.009
average	0.045	0.054	0.020	0.079	0.068	0.052	0.047	0.159	0.010
c.var	16%	40%	57%	7%	12%	15%	13%	10%	10%

Sample	Lig	Λ	S/V	C/V	%Vo	%So	Vadal	Sadal	3,5diBA /V
Sample 1	0.204	13.600	0.300	0.060	21.1	27.5	0.467	0.375	0.061
Sample 2	0.223	14.885	0.293	0.060	20.2	27.4	0.490	0.466	0.062
Sample 3	0.244	16.267	0.313	0.064	20.5	28.9	0.525	0.499	0.074
Sample 4	0.192	12.802	0.288	0.066	15.7	22.3	0.484	0.462	0.041
average	0.216	14.388	0.299	0.062	21.4	25.9	0.492	0.450	0.059
c.var	11%	11%	4%	5%	13%	11%	5%	12%	23%

or slightly higher than those obtained for lignin data. Furthermore, the determination of the n-FA concentration was not corrupted by the use of GFC filters (Appendix A).

The investigation of GFC samples reproducibility was conducted in an attempt to understand difficulties encountered with this type of sample. Details of this study can be found in Appendix A. Results showed that GFC filters are responsible for lower percentage recovery and increased degradation index $(Ad/Al)_v$, therefore forbidding its use as tracer of lignin decomposition. However, other lignin and non-lignin parameters showed no sensitivity to GFC filters and displayed reproducibility comparable to those of homogenized solid samples. A careful study of the results from samples of divers origins as well as tests conducted on blanks and solid samples seemed to attribute this problem to the composition of the filters $[Si(OH)_4]$ and the quantity of this material in the reaction medium (matrix effect).

2.3.2.3 Conclusion on the CuO technique reproducibility

The tests previously described resulted in constraining the reproducibility of the CuO technique and revealed several features:

1. The coefficient of variation of lignin phenols measurements for solid samples ranged from 4 to 8% (13% for C data) and only slightly exceeded that of just the GC-FID analysis (1-10%). Results are similar to those of Goni (1992) who found an overall variability of 2-15% in individual CuO reaction products. For n-FA this variability reached ~ 12%.

2. Poorer precision (~13%) was obtained for C parameters found in lower concentration. This feature was also noticed by Goni (1992) who observed that, in general, low compound concentration resulted in poorest reproducibility with c.var reaching as high as ± 50% for individual compounds with yields < 0.005 mg/100mgOC.

3. The degradation index (Ad/Al)$_v$ is compromised by the collection of samples on GFC filters. Possible explanations include a matrix effect during the oxidation/extraction phase probably linked to the composition of the filter itself (details in Appendix A).

4. Results for individual phenol concentration, total lignin content and n-FA of samples on GFC filters did not show particular sensitivity to the collection medium and can therefore be used for geochemical purposes.

2.4 USE OF THE CUPRIC OXIDE OXIDATION TECHNIQUE

The CuO oxidation technique involves a 3 hr, alkaline, high pressure, high temperature oxidation, followed by a chemical workup phase (details in Section 2.2.4). During this process macromolecules such as lignin are broken into smaller molecules that can be characterized and quantified by gas chromatography. The technique also gives results for non-lignin compounds such as carbohydrates, phospholipids and proteins. Whereas lignin phenols are obtained from vascular plant debris, some phospholipids, carbohydrate and proteins originate from phytoplankton-derived OM (Goni and Hedges, 1995). The CuO technique can therefore in principal be used to simultaneously measure autochthonous and allochthonous contribution to OM in a sample.

Among the numerous lignin phenols (monomers and dimers) produced during the CuO oxidation (Goni and Hedges, 1990 a; Goni and Hedges, 1990 b; Goni, 1992; Goni and Hedges, 1992; Goni et al., 1993), eight vanillyl, syringyl and cinnamyl phenolic monomers were used to assess the OM contribution from the land (Figure 1.2). The compounds used to trace the autochthonous production were chosen based on their proposed chemical precursors and biochemical sources (Goni, 1992; Goni and Hedges, 1995, Table 1). They included: (1) three n-fatty acids originating from phospholipids (found in algal membranes) and (2) two diacids and one phenol from

proteins and polysaccharides. The three fatty acids are tetradecanoic acid (n-C14FA), hexadecenoic acid (n-C16:1FA) and hexadecanoic acid (n-C16FA). The two diacids and one phenol are butane-1,4-dioic acid (C4DA), 2-butene-1,4-dioic acid (C4:1DA) and p-hydroxyphenylglyoxylic acid (Pg).

Following CuO oxidation, quantitative and qualitative information were obtained by gas chromatographic analysis. Quantitative information (i.e., concentrations: Section 2.2.7) was obtained from the integrated area of peaks corresponding to each of the compounds (lignin and non-lignin) just identified (Figure 2.2). Total lignin content was calculated as the sum of the eight V, S and C phenols concentrations. Qualitative information was obtained from the relative proportion of certain peaks to one another. This only involved lignin compounds and resulted in the determination of two important qualitative properties of land-derived OM: the type of vascular plant that contributed it and its level of degradation.

Because lignin is found solely in vascular plants, its yield, composition and relative composition are specific to each species of plants. Especially, the relative composition of syringyl to vanillyl phenols (S/V) and cinnamyl to vanillyl phenols (C/V) allows the four vascular plant tissue types (angiosperm and gymnosperm woody and non-woody tissues) contributing OM to be semi-quantitatively constrained (Figure 2.6). However, data must be interpreted with respect to the sample's level of degradation. The main decomposer of lignin on land, white rot fungi does not decompose phenols at the same rate (Hedges et al., 1988 b). Precisely, they are decomposed in the order: C>>S>V. The reason is that C phenols have ester linkages to lignin, which are more labile than the C-C and ether bonds that link V and S phenols within lignin. Therefore, while lignin content decreases with degradation, its relative composition is likely to be modified. The result of fungi degradation on a S/V vs. C/V plot is a drift of the data towards the origin, potentially corrupting the evaluation of the plant species involved. Ultimately, with time, any type of vascular plant tissues submitted to white rot fungi degradation would resemble to gymnosperm wood (G).

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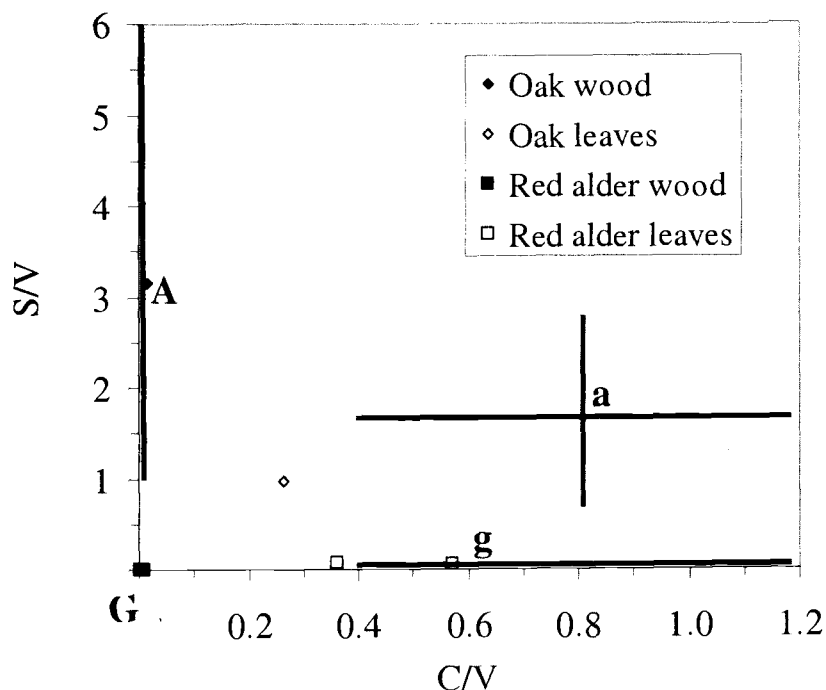
Figure 2.6 Types of vascular plant tissue source of OM to the Columbia River
Oak data: from Goni (1992). Red alder data: Goni (1992) and this study. Ranges of
value: Hedges and Mann (1979b) and Hedges et al. (1984). A, a: woody and non-
woody angiosperm tissues. G, g: woody and non-woody gymnosperm tissues

The second qualitative piece of information obtained relates to the level that
vascular plant tissues contributing to OM is degraded. A laboratory experiment
monitoring Birchwood degradation by white rot fungi clearly showed that the relative
composition of acid to aldehyde for vanillyl and syringyl phenols (Figure 2.7 a)
linearly increases from a minimum 0.15 ± 0.05 (Hedges et al., 1982) for fresh
vascular plants to higher values as decomposition proceeds. Meanwhile the total
lignin content of the sample decreases (Figure 2.7 b). It is therefore important to
associate the lignin content of a sample to its level of degradation as these two
parameters are intimately linked.
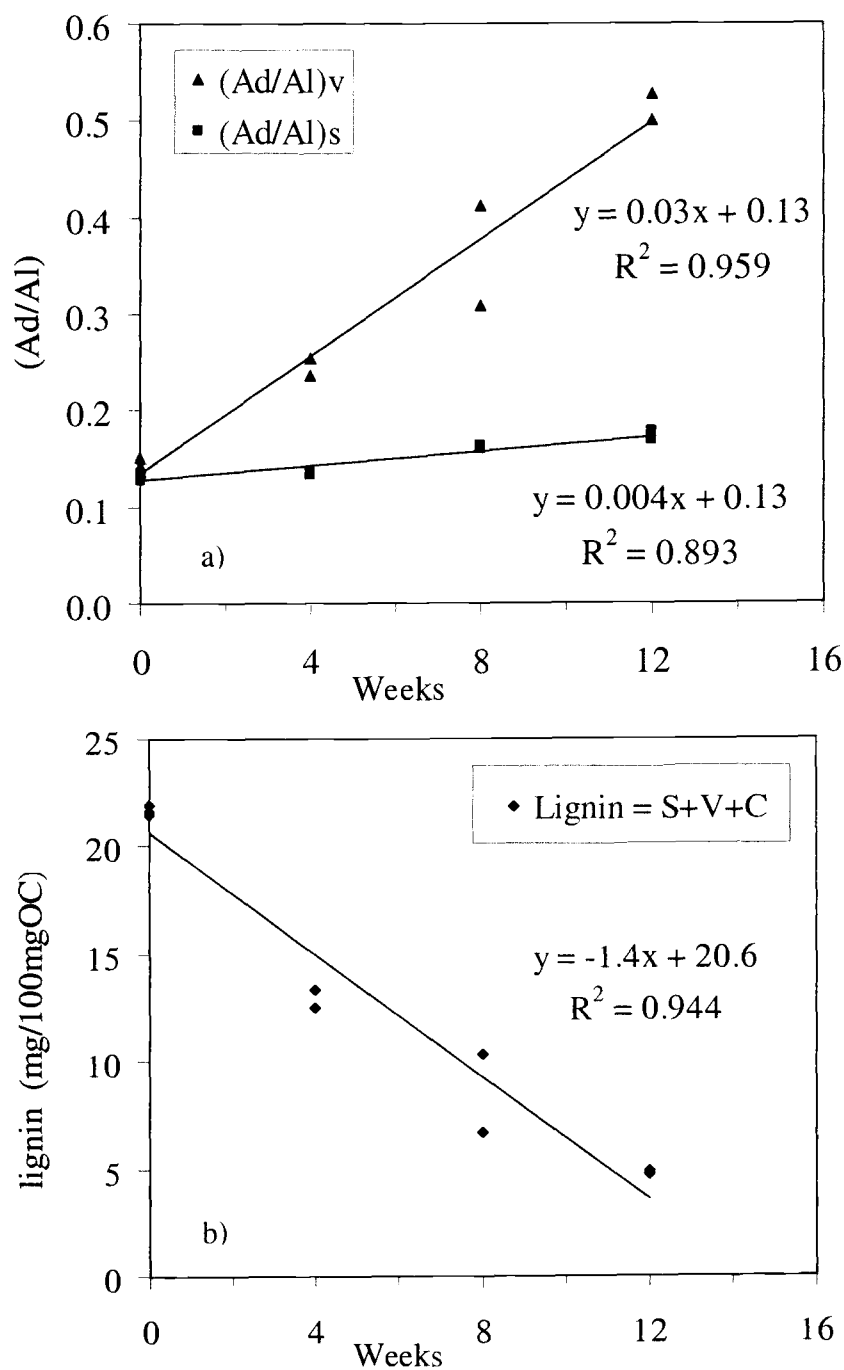
Figure 2.7 Laboratory degradation of Birchwood by white rot fungi
Results from Hedges et al. (1988b) show a) the positive correlation between $(Ad/Al)_v$ and level of decomposition (i.e., degradation time) of the wood and b) the negative correlation between total lignin content of the sample and degradation time

3: RESULTS

A major feature of the carbon cycle (Figure 1.1) is the apparent efficient recycling of land-derived OM at the land sea interface. The budgets presented were derived from world rivers. However, the organic matter content and composition in SPM from rivers could be very different from one system to another or one time to another in a given system. Given the importance of the C-cycle on earth, it would appear important to understand where and how the OM is recycled in a given system and the dependence of the processing mechanism on how the quality and quantity of the input may change with season owing to natural or anthropogenic causes.

An earlier study of the Columbia River by Sullivan et al. (2001) investigated the seasonality of the phytoplankton production at RM53 by using $(C/N)_{at}$ and Chla/POC measurements. Based on those results, a case was built ascribing the variation of the riverborne particulate organic matter (POM) content to changes in autochthonous production. It was argued that POM transport by the river is dominated by phytoplankton contribution in spring and summer while it is dominated by soil erosion and other means of vascular plant contribution in winter. The following discussion will focus on results from the CuO oxidation technique, which provide an independent analytical eyepiece for assessing the origin, quantity, quality and seasonality of suspended POM in the Columbia River. The similarities and differences in findings from these two analytical approaches will be identified and discussed.

3.1 INTRODUCTORY DISCUSSION

The Columbia River is the largest Pacific Northwest River. Yet, with a mean annual flow of 7,500 m^3s^{-1}, its SPM concentration is lower than that of the Fraser River (64

to 175 mg/L, summer sampling, Appendix V) characterized by a mean annual flow of 2,720 m^3s^{-1}, and equal that of the St. Lawrence River (5-40 mg/L, Pocklington and Tan, 1987), whose mean annual flow reaches 10,100 m^3s^{-1}. The Columbia River contrasts the case for most rivers in temperate regions where SPM concentration is strongly dependent on river flow (Probst, 1992).

SPM concentrations in the Columbia River are never very high. They typically range from 10 to ~40 mg/L (Sullivan et al., 2001; Covert, 2001; Appendix V) and show no discernable seasonal trend [Figure 3.1 a)]. High SPM concentration is possible, but only under now relatively rare flooding events such as in February 1996 [Figures 3.1 a) and b)]. Nonetheless, riverborne SPM concentrations show a slight, direct dependence on flow (Figure 3.1 b), revealing that the system is starved for fine particles (Sherwood et al., 1990; Sullivan et al., 2001). As a result, low and high discharges typically exhibit fairly similar SPM concentrations. Flow data for the years of samples used in this thesis are displayed in Figures 3.2 a) and b).

In contrast to SPM, the OM content of the river (i.e., %POC) varies seasonally (range: 2.4-7.9, mean: 4.8, Figure 3.3 a) and can get quite high (13.2%, April 1996). However, this seasonal trend is partially masked by high variability in a given sampling period. For example, %POC ranged from 3.6 to 7.2 in RM53 bulk samples collected during a 12-hour time period on February 21st 1998. Such variability results from the decrease of %POC with SPM increase (Figure 3.3 b) and the slight increase of SPM with flow. When compared to other rivers, the Columbia River %POC is typically much higher than that of the Fraser River (range: 0.8-1.9, mean: 1.3) and twelve North American Rivers east of the Rockies (range: 0.7-3.8; mean: 1.9, Canfield et al., 1997; Onstad et al., 1998), and compares most closely with the St. Lawrence River (range: 3-14, mean: 7, Pocklington and Tan, 1986). On a global perspective, the Columbia River %POC is higher than the average for world rivers (1%, Meybeck, 1982). Historical data on the Columbia River SPM and POC content would be interesting as they would show if the actual high %POC observed is a normal characteristic of the river or an artifact of recent human management.
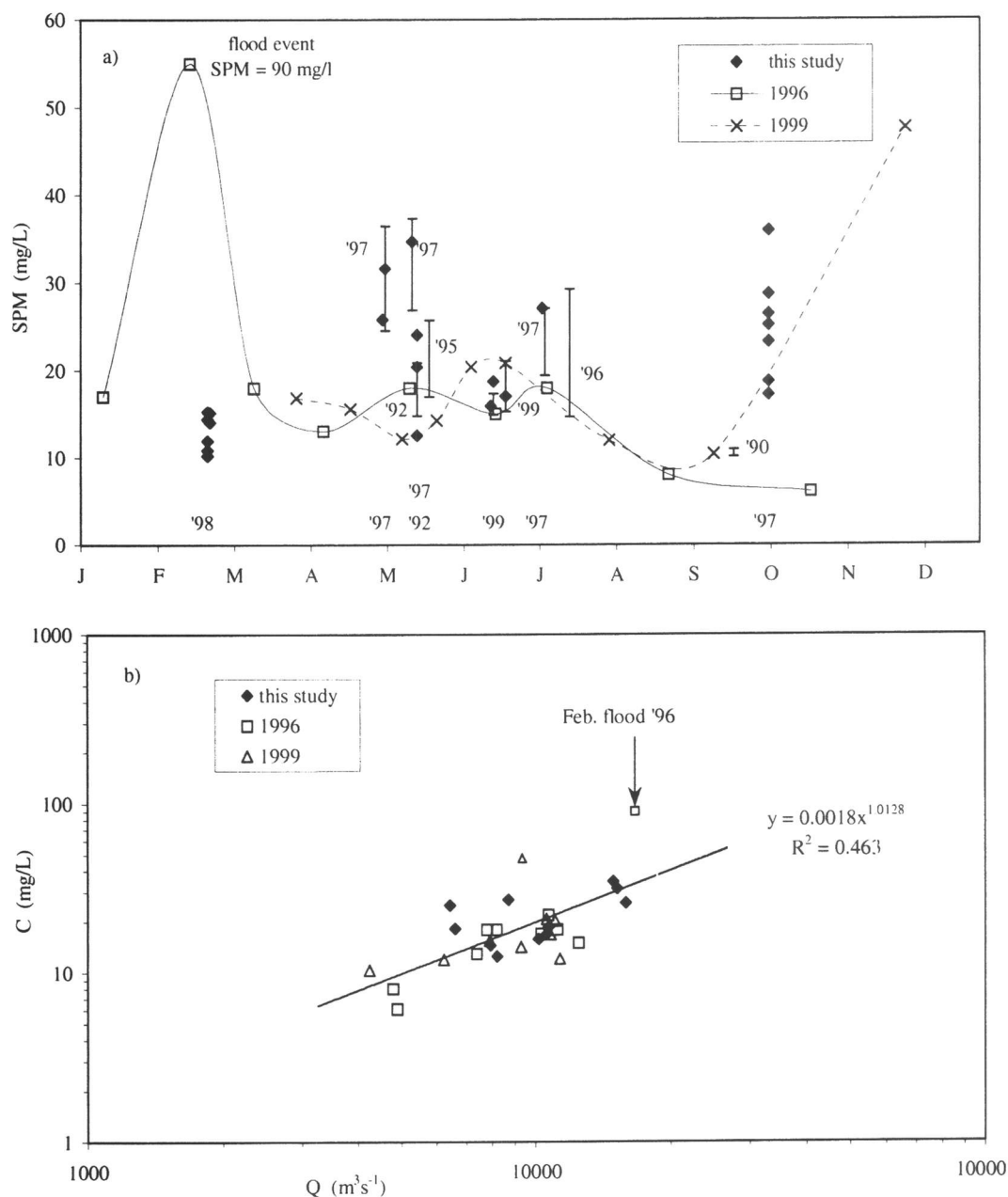
Figure 3.1 Columbia River SPM seasonality and flow dependency at RM53
(a) SPM seasonality; (b) SPM changes with river flow. 1999 and 1996: cross-channel
isokinetically collected samples, Covert (2001) and Sullivan et al. (2001)
respectively. This study: mid-water column samples [years reported at bottom of plot
(a)]. Error bars are the variation about the mean measurements of other LMER
sampling years (1990-1999). C: SPM concentration (mg/L). Q: discharge (m^3s^{-1})

... in the western subbasin: episodic high flow	High flow from snowmelt in eastern subbasin	Lowest flow of the year (dry season)	Increase of rainfall and runoff
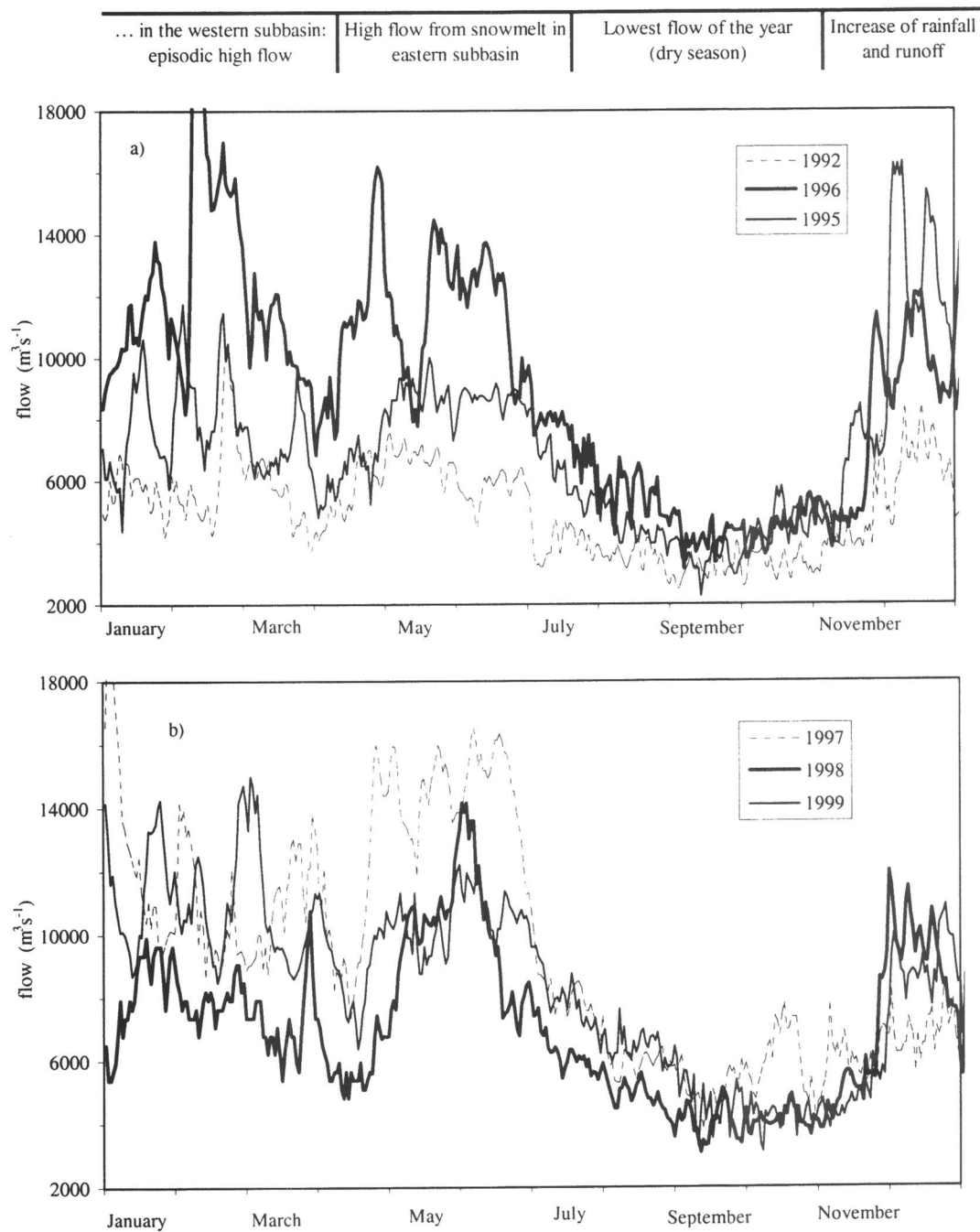
Figure 3.2 Seasonality of Columbia River flow at RM53
(a) 1992, 1995 and 1996 and (b) 1997, 1998 and 1999. Data from USGS website:
http://waterdata.usgs.gov/or/nwis/discharge

The %POC and SPM concentration for Columbia River, Fraser River and twelve North American rivers (Canfield et al., 1997; Onstad et al., 1998) are related in a conspicuous fashion [Figure 3.3 b)]. This feature, characterized by low %POC (~ 1 %) in rivers of high turbidity (SPM > 1000 mg/L) and high %POC (10-15 %) in rivers of low turbidity (~ 10 mg/L), was first noted by Meybeck (1982) and further discussed in many other studies (Ittekkot, 1988; Probst, 1992; Ludwig et al., 1996; Sullivan et al., 2001). Two different biogeochemical processes could account for the nonlinear decrease of %POC with increasing SPM concentration. In rivers such as the Loire (Meybeck et al., 1988) and St. Lawrence (Pocklington and Tan, 1987), significant phytoplankton production occurs. The %POC decrease with increasing SPM therefore reflects a dilution of autochthonous organic matter with relatively organic matter-poor mineral mass. In more turbid, systems such as the Amazon River (Hedges and Clark, 1986; Devol and Hedges, 2000), allochthonous OM (vascular plant, soil) dominates. The decreasing trend is caused by a shift in input from organic-rich litter and topsoil to mineral-rich soil from deeper horizons (Desjardins et al. 1991) as a result of the intensification of mechanical erosion.

To identify which of the two previous processes drives the trend between %POC and SPM in the Columbia River, information about POC source contribution is necessary. Sullivan et al. (2001) attributed POC increase at low SPM to the presence of a significant phytoplankton biomass.
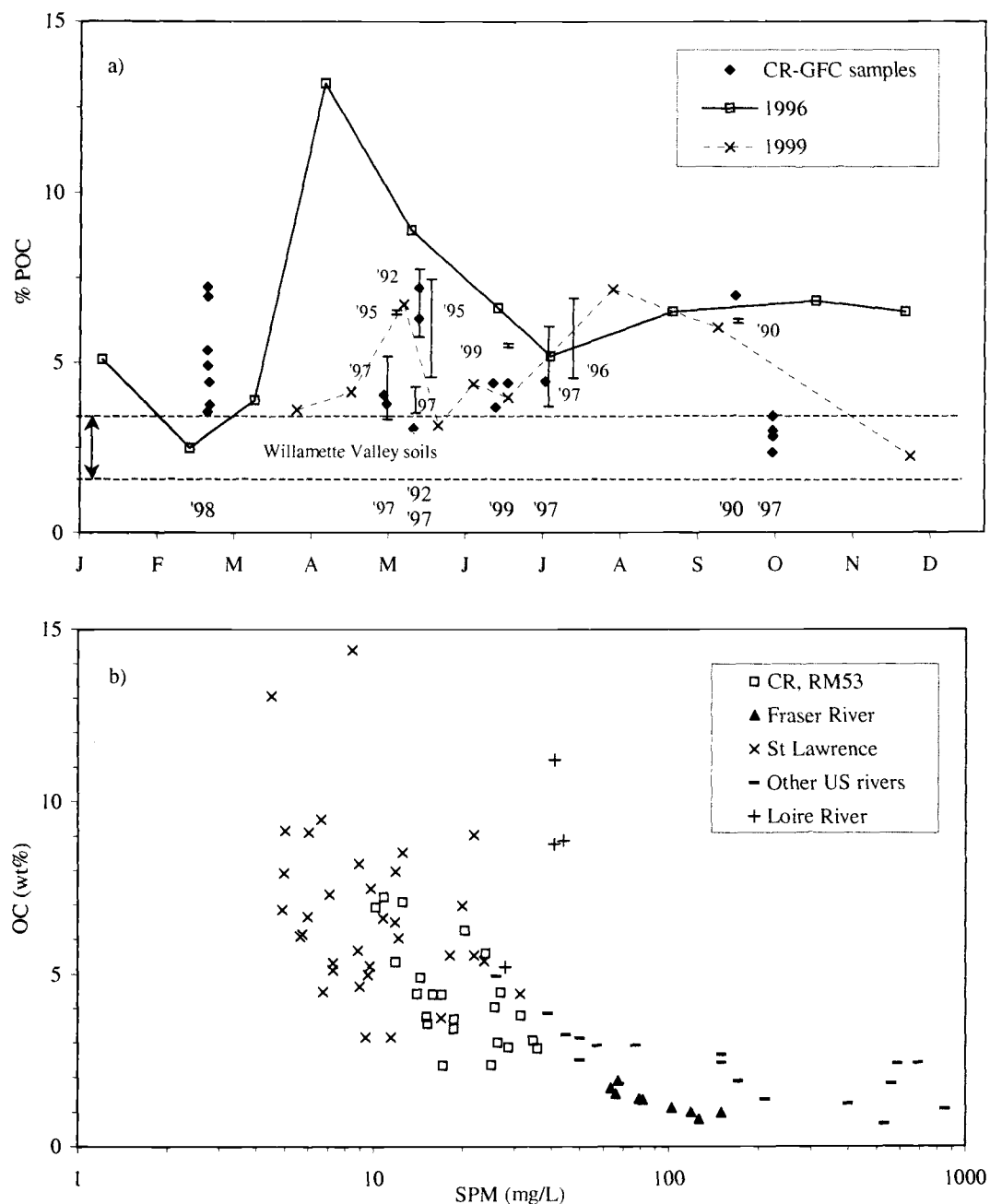
Figure 3.3 Columbia River POC properties
(a) POC seasonality. 1999: Covert (2001); 1996: Sullivan et al. (2001). CR-GFC
samples collected in 1992, 1997, 1998 and 1999 (years reported at bottom of plot)
and analyzed for lignin content. Error bars are the variation about the mean of other
LMER sampling years (1990-1999). (b) Comparison of trend for POC weight
percentage versus SPM concentration in Columbia River samples with that for the St
Lawrence (Pocklington and Tan, 1987), Loire (Meybeck et al., 1988) and other rivers
located in the contiguous US east of the Rockies (Canfield, 1997).

3.2 SOURCES OF OM TO THE COLUMBIA RIVER

Results from my use of the CuO oxidation technique are presented and discussed as an independent analytical eyepiece for assessing the origin of suspended OM in the Columbia River (RM53) and how it may change as a function of varying river conditions (natural and anthropogenic). Consistencies (and inconsistencies) of this assessment versus that made by alternative methods (PN/POC and Chla/POC) are identified. The complete dataset is displayed in Appendix V and includes SPM concentration, POM content and composition (%POC, C/N, Chla and CuO oxidation products).

3.2.1 Assessment of OM Quality and Origin

A time series plot of Chl/POC measured in samples from this study [Figure 3.4 a)] provides further evidence for the importance of phytoplankton contribution to POC in the modern Columbia River. Throughout the year, values for almost all the samples fall within a range (10-30 mg/gOC) considered representative of 'healthy' phytoplankton (Dietrich, 1977; Harris, 1986; Meybeck et al., 1988; Riemann et al., 1989; Descy et al., 1994). Figure 3.4 a) shows the same seasonal pattern as that found by Sullivan et al. (2001) although not as detailed. Low phytoplankton biomass is observed during fall and winter that typically are periods of low light, low nutrient availability whereas high phytoplankton biomass is apparent in spring and summer. Additional support for this interpretation of Figure 3.4 a) is the strong correlation (r^2 = 0.82) noted between $(N/C)_{at}$ and Chl/POC [Figure 3.5 a)]. The explanation for this correlation is that phytoplankton, the source of chlorophyll-a, are enriched in N relative to erosional sources of OM (e.g., vascular plant litter, mineral-associated soil humus). As phytoplankton contribution increases, the Chla and PN content in OM increases. However, this increase is not strictly linear, as Chla and PN levels in
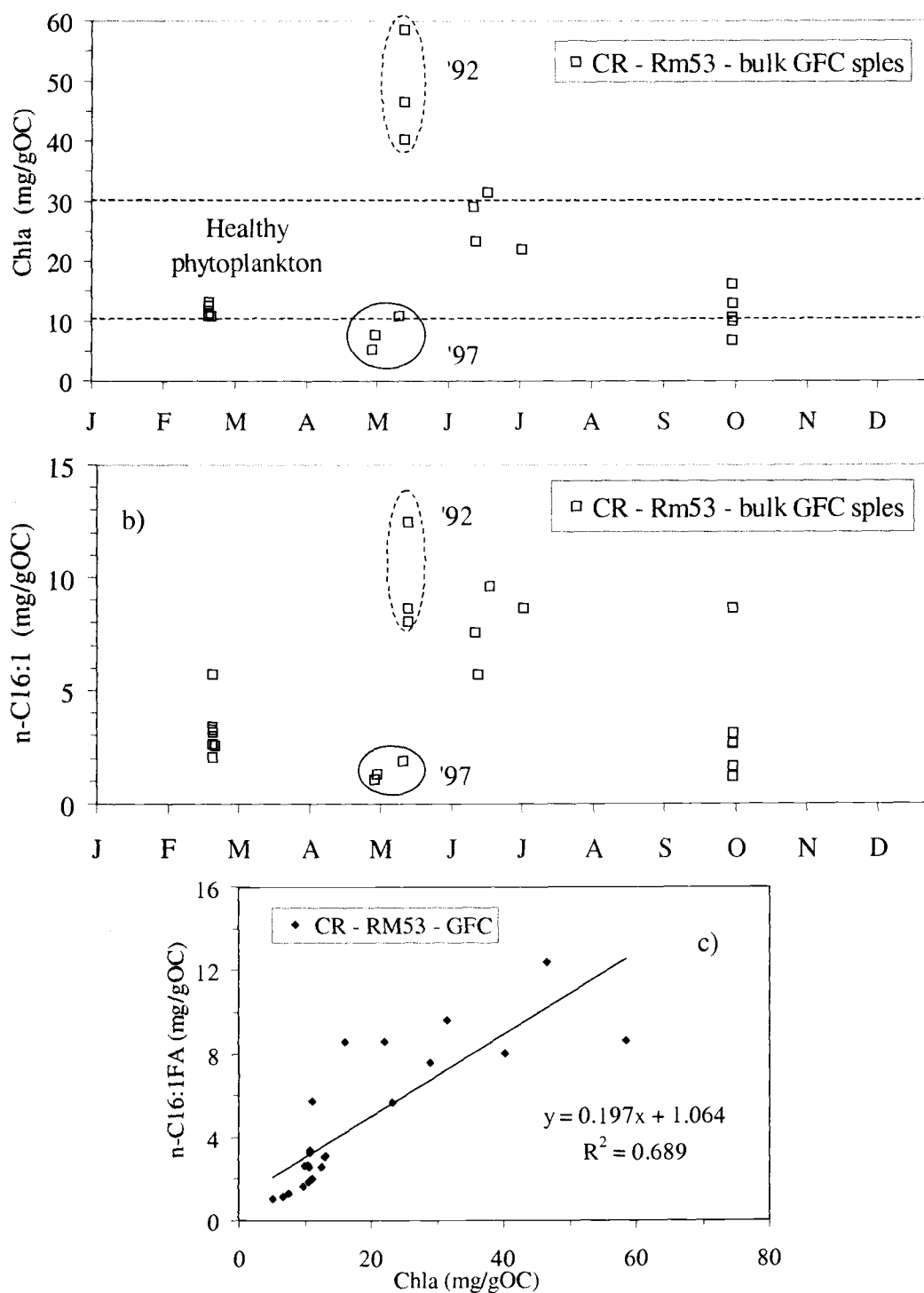
Figure 3.4 Autochthonous OM seasonality
Seasonality of a) Chlorophyll-a and b) hexadecenoic acid at RM53 for GFC samples.
1997 and 1992 data (circled) correspond respectively to high and low flow years. c)
correlation between Chla and n-FA
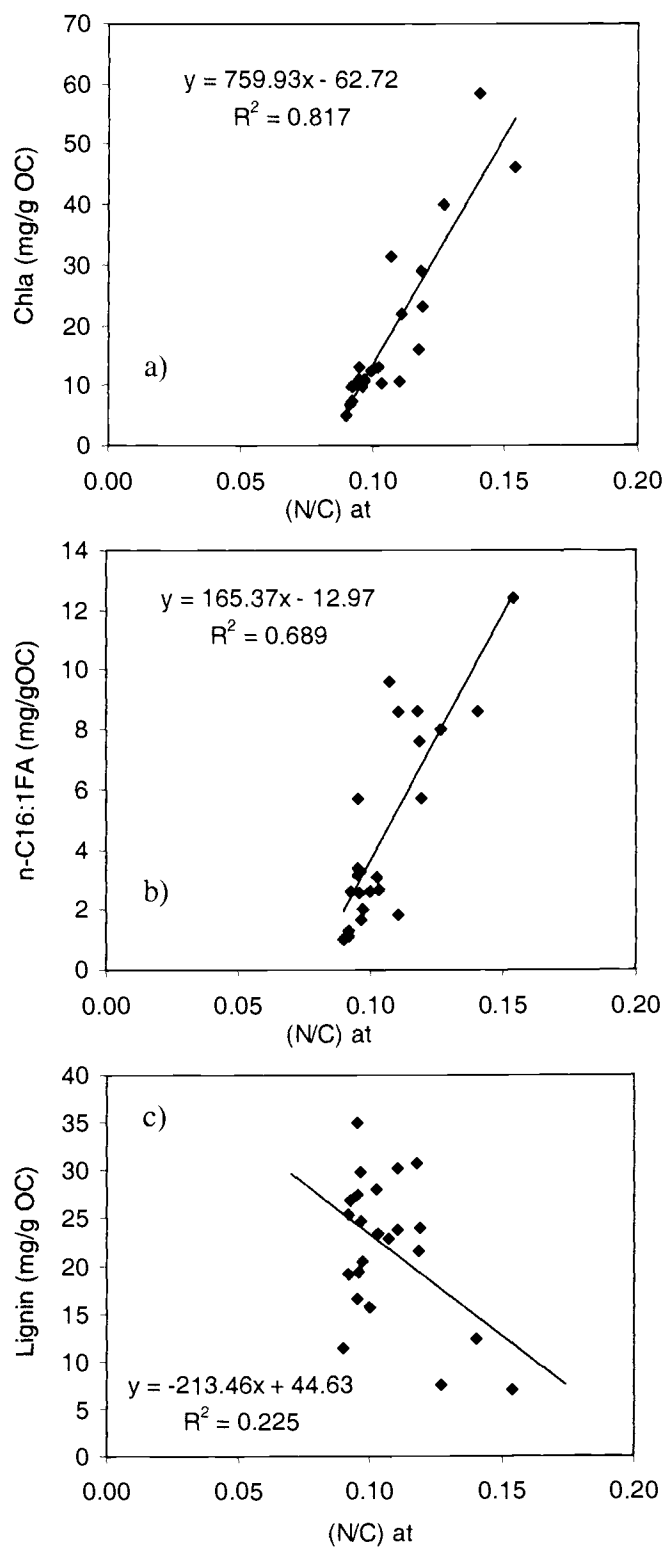
Figure 3.5 Correlation between $(N/C)_{at}$ and Chl-a, n-FA and lignin content

phytoplankton can vary depending upon physiological factors such as light and nutrient availability.

Results from n-FA ($nC_{14,16,16:1}$), CuO tracers for autochthonous OM follow a very similar seasonal pattern to that just described for Chla [Figure 3.4 b)]. Further evidence for this feature comes from the direct positive correlation ($r^2 = 0.70$) between the n-FA and both Chl-a and $(N/C)_{at}$ data [Figures 3.4 c) and 3.5 b)]. Such results are expected if phytoplankton were the dominant source of non-lignin-derived CuO oxidation products. However, other n-FA derived from proteins and polysaccharides (C4DA, C4:1DA, Pg) that were anticipated to be of phytoplankton origin showed little correlation with Chl/OC. In fact, correlation was even better (although not strong) with lignin content. Furthermore, the n-FA trend reinforces the hypothesis that the degree of phytoplankton dominance of OM during the period of spring freshet (May/June) is dependent upon river flow. Indeed, phytoplankton contribution is lower in high flow (1997) than in low flow (1992) years.

Lignin data showed a somewhat opposite trend to that of Chla and n-FA [Figure 3.6 b)]. Slightly lower concentrations were observed in spring and summer than during fall and winter. Lignin phenols were inversely related to $(N/C)_{at}$ and Chl/OC [Figure 3.5 c) and 3.6 c)], although the correlation was not particularly strong ($r^2 = 0.22$ and 0.31, respectively). Negative correlation was expected if vascular plant litter and mineral-associated soil humus, sources of lignin to the system, are N-depleted relative to phytoplankton and contain no measurable chlorophyll.

However, the interpretation of the lignin data is not as clear as that of both Chla and the n-FA data and evidence for seasonality in lignin concentration is not conspicuous. Biogeochemical explanations for the less clear message conveyed by the lignin data are possible. First, combined results from studies performed over the years have shown that quantitative interpretation of lignin data generated by the CuO oxidation method can be complicated. Lignin content of pure terrestrial organic matter varies depending upon the environmental source of input (plant tissues and species, soil type and horizon; Appendix IV and references therein) and its level of

degradation. Second, not all fresh vascular plant tissues yield lignin phenols with the same efficiency (Hedges and Mann, 1979). Sarkanen and Ludwig (1971) report that CuO oxidation yields for vanillyl and syringyl phenol from the lignin polymer differ, being 30% and 90% respectively. Consequently, carbon-normalized lignin concentrations of fresh plant materials are not constant and can vary widely (Appendix IV). Third, vascular plant material contributing OM to the river is not fresh and has likely undergone various stages of diagenetic alteration. Figure 2.7 b) clearly showed that lignin content decreases with the degradation level and that the determination of the plant types source of OM could be complicated as well. The extent of diagenetic alteration has traditionally been gauged by analysis of acid to aldehyde ratios of vanillyl phenols (Hedges et al., 1988b). However, analysis of samples collected on GFC filters in my study has compromised this evaluation (Appendix A). Finally, the study of two 12 hours time series (Appendix B) suggests that lignin content of SPM varies on a daily basis owing to tide events. Factors such as river flow, resuspension of material from the riverbed and season (i.e., environmental factors) appears to influence greatly both the quantity and the origin of the OM found in suspended material (degradation could not be evaluated). These factors resulted in having the lignin concentration over 12 hours to span the same range of values as seen throughout the year. Therefore, collection time is a crucial parameter when investigating lignin seasonality in the Columbia River. The sampling strategy was not adapted to this feature, which potentially resulted in masking the actual seasonality of the lignin content at RM53. None of these factors are taken in consideration in Figure 3.6 b.
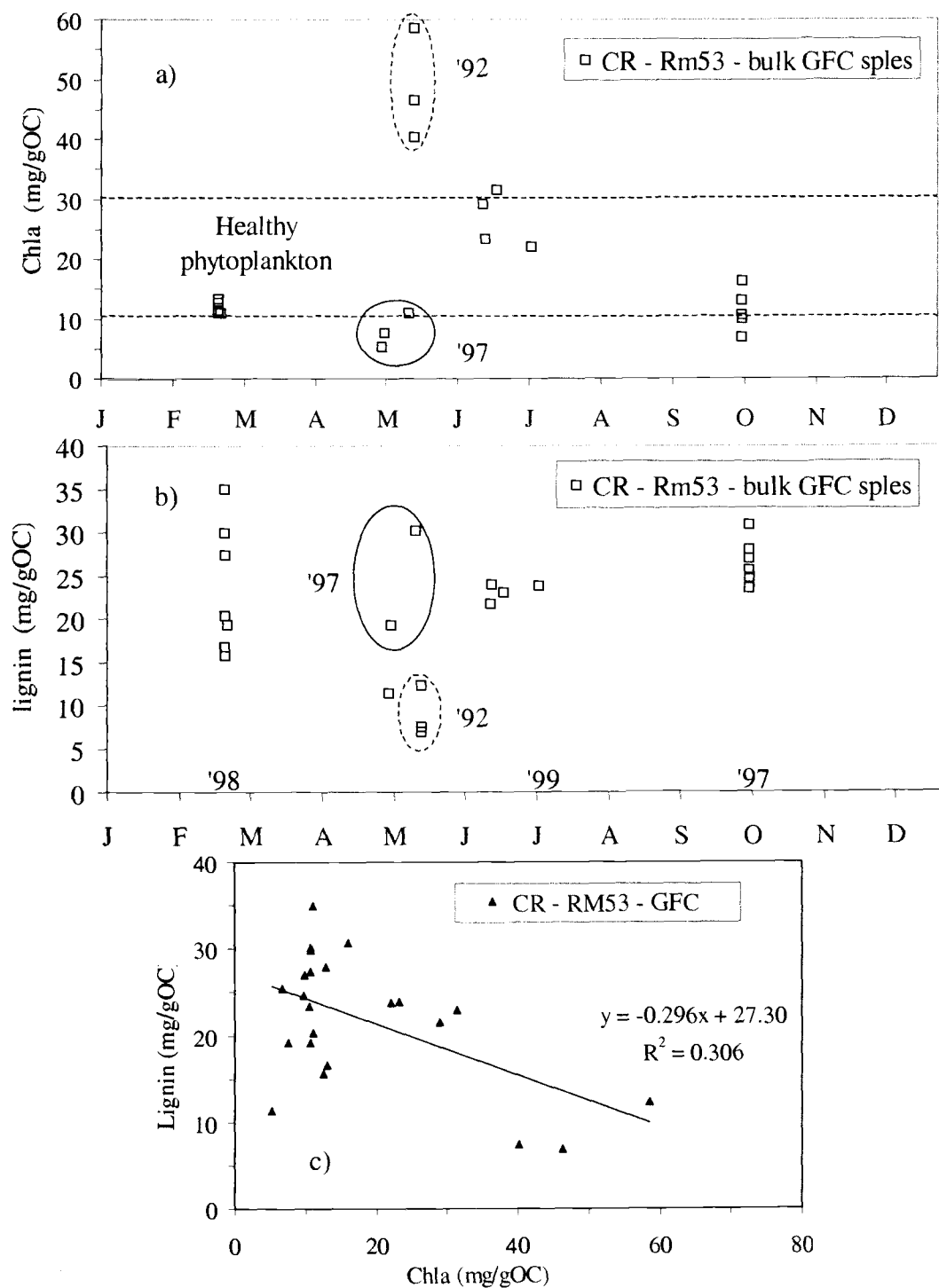
Figure 3.6 Seasonality of allochthonous OM
b) lignin seasonality at RM53, GFC samples. c) correlation between lignin and Chla

3.2.2 Sources of Lignin

Because lignin is produced exclusively by vascular plants (Sarkanen and Ludwig, 1971), its yield (Λ, mg/100mgOC) and composition (S, V, C phenols) upon CuO oxidation are indicative of terrestrial plant sources. Indeed, each species is defined by specific values for S/V and C/V. A property-property plot of these ratios can be used to delineate four broad categories of plants: woody and non-woody gymnosperms and angiosperms tissues (G, g, A, a respectively). In the past 20 years, extensive studies have determined the dominant input of vascular plants (Hedges and Mann, 1979 a; Ertel and Hedges, 1985; Goni and Hedges, 1990 a and b; Goni and Hedges 1992; Appendix IV) to soils (Prahl et al., 1994; 1997), marine (Hedges and Mann, 1979 b; Ertel and Hedges, 1984; Ertel and Hedges, 1985; Prahl, 1985; Prahl et al., 1997; Keil et al., 1998;) and river (Hedges et al., 1984) sediments of the Pacific Northwest. These earlier results are used in the S/V vs. C/V plot (Figure 3.7) to determine the origin of the lignin input to the Columbia and Fraser Rivers.

A first look at the large-scale S/V vs. C/V plot [Figure 3.7 a) and c)] shows that the overall lignin signature in the two rivers and in the Columbia River estuary are quite similar. Lignin is derived predominantly from woody and non-woody gymnosperm, with yet a noticeable contribution of non-woody (and woody) angiosperm tissues in the Columbia River (G>A~g>a). These results agree with data obtained for local vascular plant species and soils (Appendix IV) as well as with earlier results from Columbia River (Hedges et al., 1984; Prahl et al., 1994) and marine sediments (Figure 3.9) from the Washington Continental shelf (Keil et al., 1998). This is not surprising as the Columbia River is by far the dominant source of the sedimentary material deposited in the coastal region (Nittrouer, 1978). Results are also consistent with the fact that decaying leaf material, rather than woody debris, constitutes the major portion of litterfall (Duke et al., 1981; Heald, 1971).
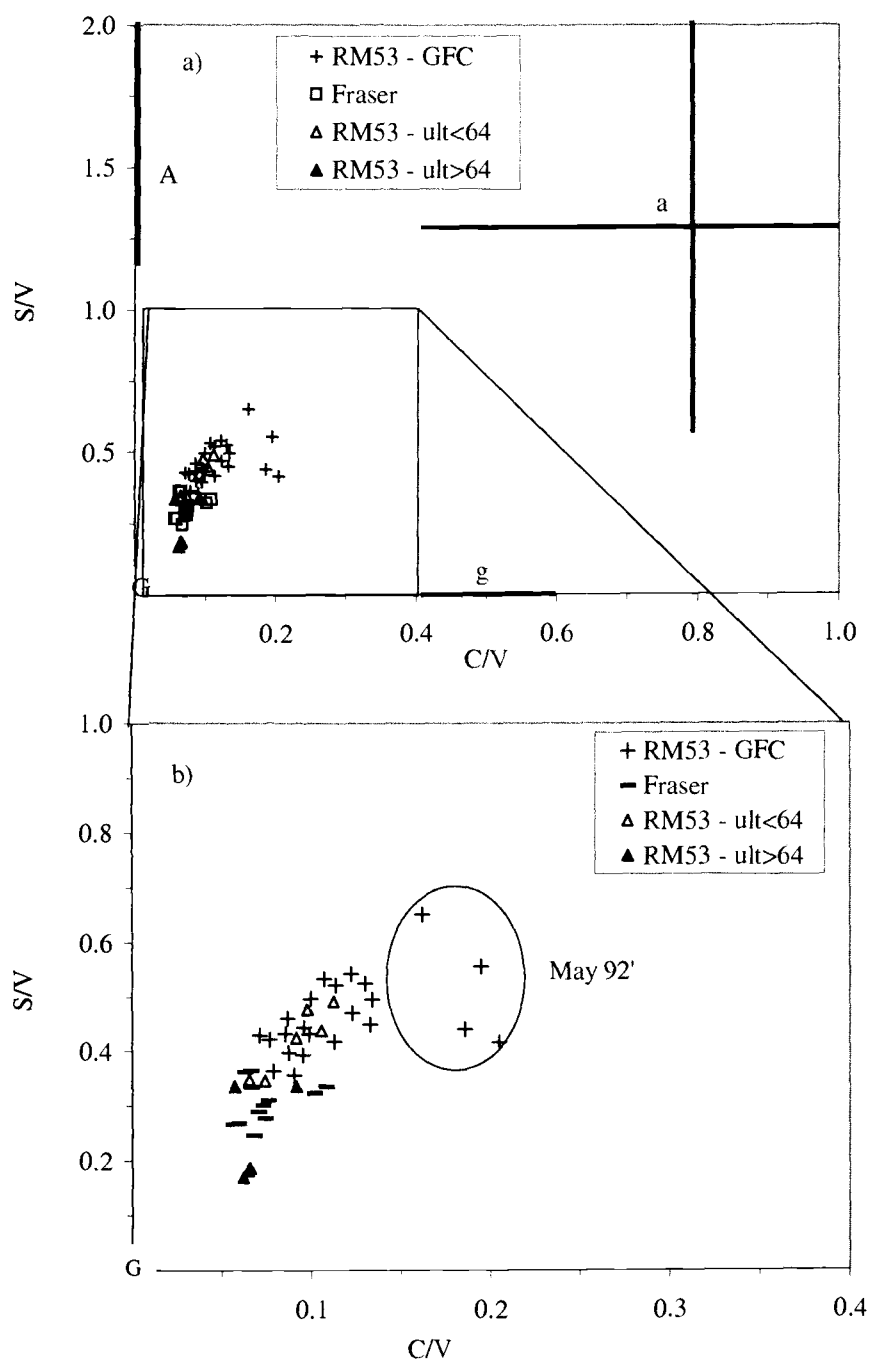
Figure 3.7 Sources of lignin to the systems: (S/V) vs. (C/V) plot

Plot (a) shows boundaries for species end-members: (G) woody and (g) non-woody gymnosperm, (A) woody and (a) non-woody angiosperm. GFC: bulk samples; ult: size-fractionated samples from the Columbia (RM53) and Fraser Rivers. Plot (b) is a blow-up of plot (a). Ranges are from Hedges and Mann, 1979b; Hedges et al., 1984; Keil et al., 1998
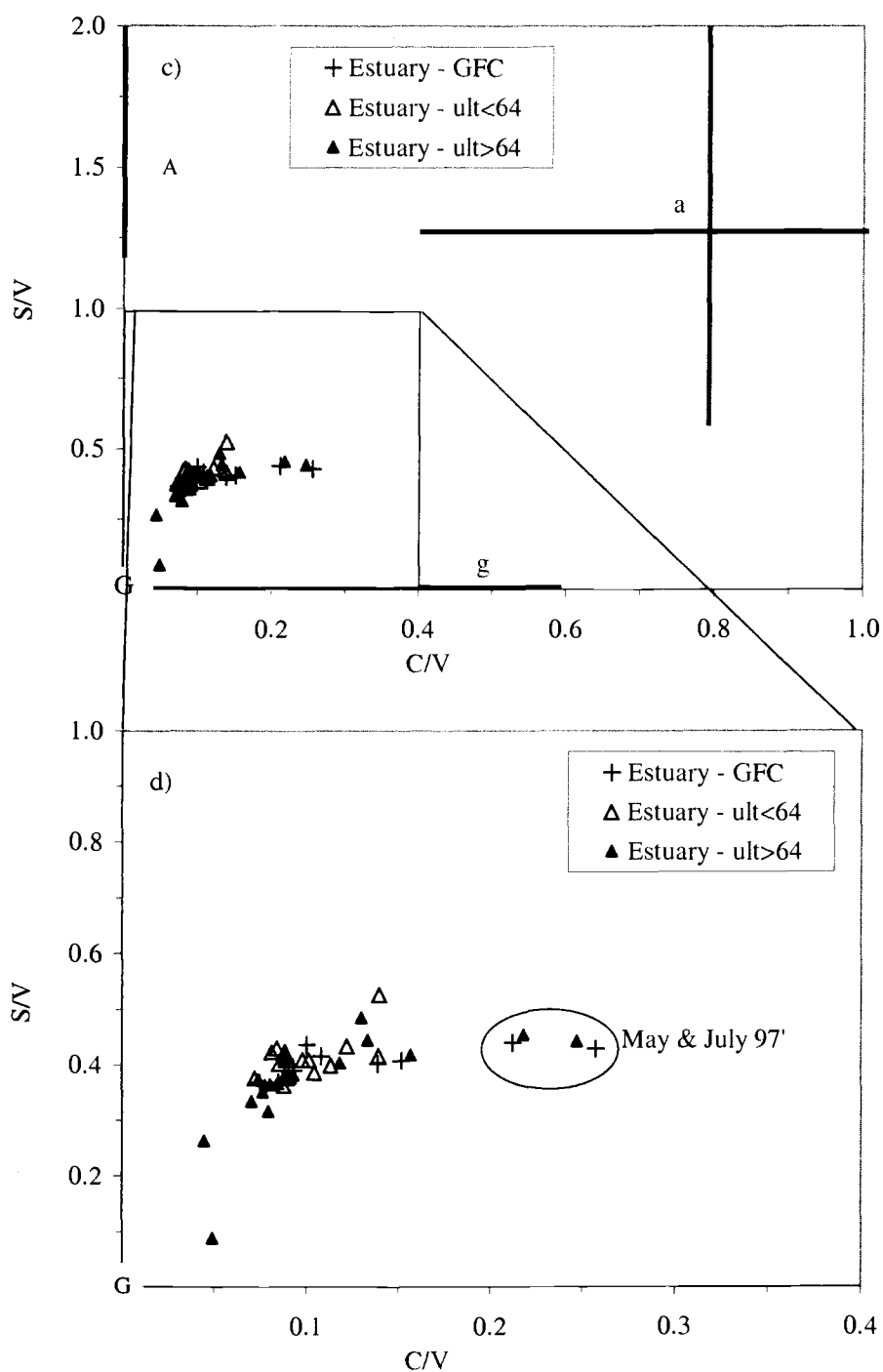
Figure 3.7 Continued

Plot (c) shows boundaries for species end-members: (G) woody and (g) non-woody gymnosperm, (A) woody and (a) non-woody angiosperm. GFC: bulk samples; ult: size-fractionated samples from the Columbia estuary. Plot (d) is a blow-up of plot (c). Circled data in plot (b) and (d) are spring freshet samples: Mai-June 1997

Closer inspection of these plots [(Figures 3.7 b) and d)] reveals interesting features that may be associated with sample grain-size and collection sites. The first feature shows compositional differences between coarse (> 64 μm) and fine (< 64 μm) Columbia River samples (Figure 3.7 b). Fine particles, which compose the majority of the SPM (Figure 8), contain a higher amount of non-woody angiosperm (and gymnosperm) tissues than the coarse particles, which are more like woody-gymnosperm. However, ratios are low relative to fresh angiosperm material indicating that the fine fraction either has an overall strong woody gymnosperm component or that its OM has undergone high degradation. An earlier study of the Washington coast sediments (Keil et al., 1998) reports the same observation about particle size and lignin sources.
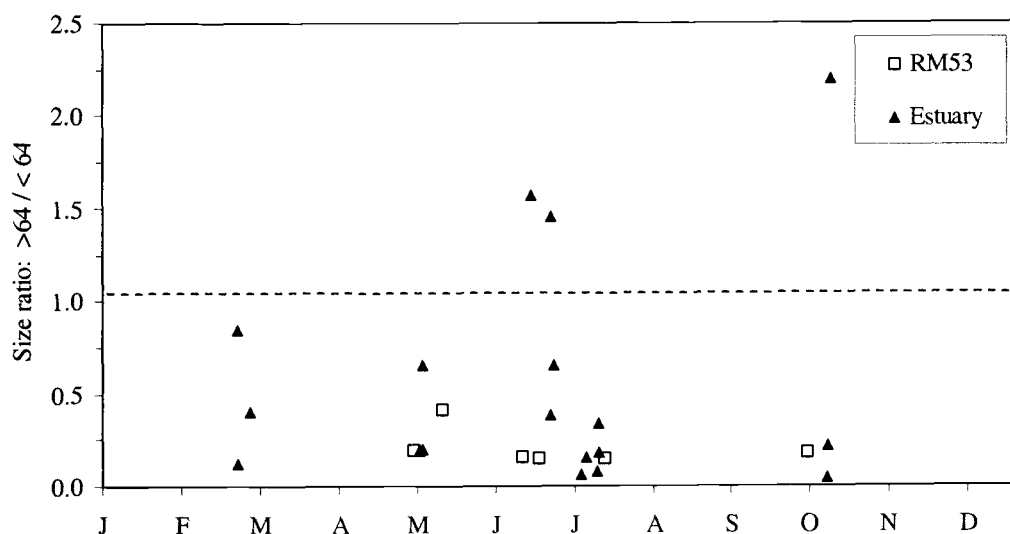
Figure 3.8 Size distribution of Columbia River SPM
Size ratio between coarse and fine SPM fractions at RM53 and estuary. In both location fine particles dominate the load with the exception of three estuary data for June 1999 and October 1997

The second aspect concerns Columbia River estuary samples, which interestingly showed the same overall size-fractionated characteristics mentioned earlier for river samples (Figure 3.7 d). In this case however, fine particles were not always the

dominant component of the estuary suspended load (Figure 3.8). This absence of compositional difference between GFC and size-fractionated samples from RM53 and the estuary was surprising and potentially implied that no size-based segregation (i.e., physical sorting) happened within the estuary [Figures 3.7 b) and d)]. This was surprising given that selective particle trapping was observed in earlier studies (Gelfenbaum, 1983; Simenstad et al., 1994; Reed et al., 1994) of ETM where these samples were collected. Reed et al. (1994) observed differences in particle size, organic content and settling characteristics between flood and ebb ETM. ETM occurring during ebb tides seem to be triggered by advection (resuspension) of particles from the riverbed, where those occurring during flood tide seem to be driven by selective particle trapping. The likely explanation for my results is that the estuary data reported in Figure 3.7 were collected during different tide and flow regimes as well as during different months and years. As SPM characteristics (quality and quantity) of ETM vary on a short (daily) and long (seasonal) basis, grouping all the data collectively resulted in masking the variability, leading to similar results for the estuary and river samples.

Finally, the Columbia and Fraser River GFC samples display slight differences in composition. The Fraser River seems to contain more gymnosperm wood than the Columbia River, which is relatively richer in A and non-woody tissues. These observations agree with the vegetation found locally within the two rivers basins. Whereas the Fraser River basin is dominated by coniferous forest, the east side of the Columbia River catchment includes vast arid steppe regions covered by grasses and herbs and almost devoid of trees (Atlas of the Pacific Northwest, Oregon State University Press, 1993). As a result, sediment samples from Crab Creek and Long Lake Reservoir on the Spokane River (Figure 3.9) are particularly rich in syringyl and cinnamyl phenols (Hedges et al., 1984; Prahl et al, 1994), characteristic of angiosperms. The input of non-woody angiosperm tissues happens principally during spring freshet (typically May), when a considerable amount of water from the eastern subbasin enters the system due to snowmelt (Sherwood et al., 1990). This feature is

particularly well-documented by the two groups of data circled in Figures 3.7 b) and d) that correspond to samples from spring (May) 1992 and 1997.
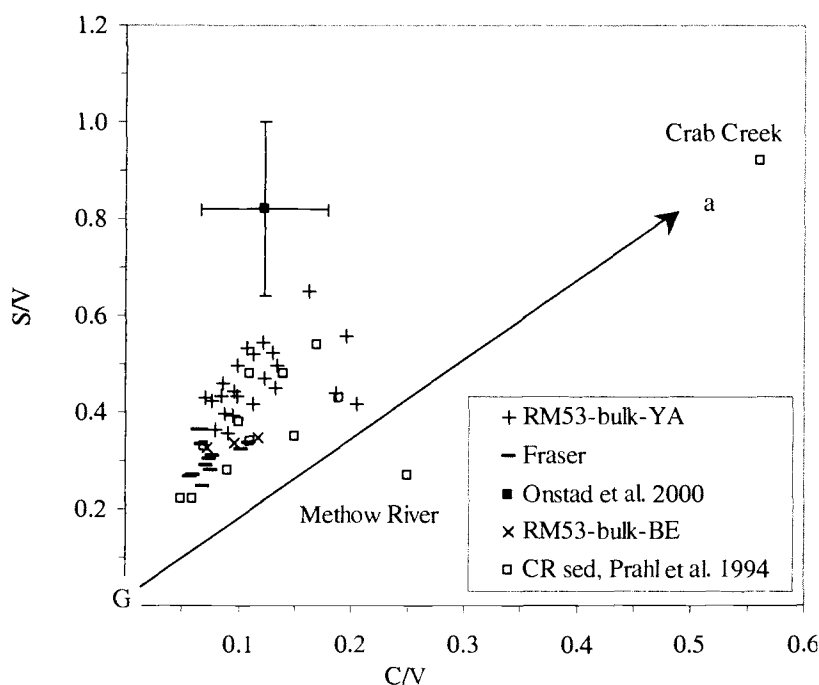
Figure 3.9 Lignin sources in the Columbia River
Onstad et al. (2000): data for 12 continental US rivers. Prahl et al. (1994): Columbia River sediments. b)

3.2.3 Columbia River vs. Other Large US and Canadian rivers

An important environmental characteristic of the Columbia River is that its SPM is typically enriched in organic carbon (Figure 3.3 b) relative to the Fraser River and other North American rivers. Its OM is also of different quality than that carried by the other rivers in that it exhibits higher concentrations of PN on average (Figure 3.10). $(C/N)_{at}$ in the Columbia River averaged 8.7 (range: 6.4 to 11.1, Appendix V), which is close to 7 expected for phytoplankton (6.6: Redfield et al., 1963; 5-8: Hedges and Oades, 1997 b; Hedges et al., 1997 a). In periods of low flow conditions such as May 1992 (Figure 3.2), $(C/N)_{at}$ averaged 7.1 possibly implying that most or

all of the OM was autochthonous. However, the annual average value of 8.7 implied mixing with soil OM whose $(C/N)_{at}$ typically ranges from 10 to 13 (10: Bowen, 1979; 8-12: Stevenson et al., 1972; 11.1-13.4 and 13.7 respectively for Willamette basin deciduous and coniferous forest, Prahl et al., 1994; 9.3-10.6, Amelung et al., 1997; 7-15: Hedges and Oades, 1997 b).

As a comparison, POM in the Fraser River (summer values) is much more N-depleted, with $(C/N)_{at}$ averaging 22.4 (range: 19.3-40.6) and somewhat more N-depleted than that of twelve other North American Rivers (range: 9.2-13.4; mean: 11.3, Onstad et al., 1998, summer values) including the St. Lawrence (range: 8.5-12.7; mean: 10.4, Pocklington and Tan, 1987). The Fraser $(C/N)_{at}$ data corresponds to the lower end of values expected from fresh terrestrial vascular plant tissues (20-400,
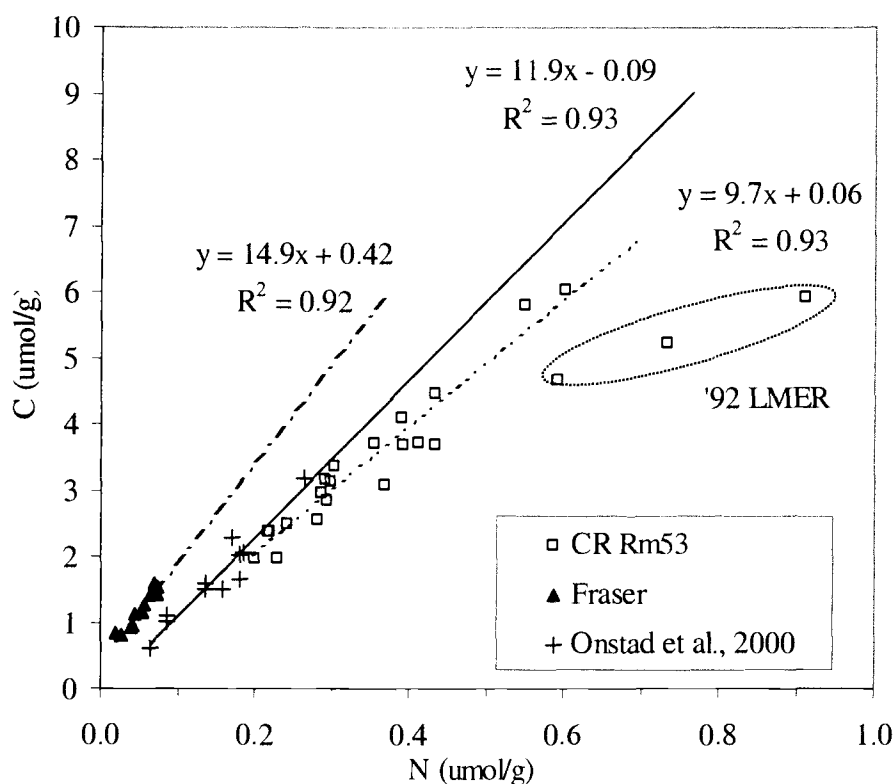
Figure 3.10 Concentration of POC versus PN (μmol/g)
Plots imply that all of the nitrogen is of organic origin

Hedges et al., 1986; Hedges et al., 1997) whereas those from the other North American Rivers are typical of soil OM. These systems appear dominated by land-derived OM input, the Fraser River receiving a greater quantity of fresh vascular plant debris or input from upper most soil horizons (Hedges and Oades, 1997; Desjardin et al., 1991). The average of 9.9 for world rivers (Meybeck, 1982) indicates that the Columbia River has a higher than average input of N-rich OM, which is consistent with greater contribution of phytoplankton.

Lignin content of samples reported as Lig (mg/g dry sediment) and LIG (mg/gOC) in Appendix V provide different and complementary environmental information. Carbon normalized lignin concentrations (LIG) indicate the proportion of POC that originates from lignin while Lig reveals the proportion of the total sample mass that comes from some type of vascular plant tissue. Columbia River samples display a wide range of lignin content throughout the year from 6.4 to 35 mg/gOC, averaging 19.5 mg/gOC. Interestingly, such values are similar to those measured in the Fraser River for samples collected during summer (July 1999: range = 16.5-36; average = 21.9) and are slightly higher than that of other North American Rivers east of the Rockies (range: 4.5-31.6; average: 13.3, Canfield et al., 1997; Onstad et al., 1998). These observations are surprising given that Columbia River organic matter appeared phytoplankton-rich based on Chla, $(C/N)_{at}$ and n-FA analysis. Therefore, from a cursory standpoint, one might expect lignin content (as percentage of OM) to be much lower in the Columbia River than in the Fraser River (very low Chla content, Appendix V) and the other North American Rivers (based on $(C/N)_{at}$ data) owing to dilution by lignin-poor phytoplankton OM.

S/V vs. C/V plots suggested that gymnosperm tissues are the main land-derived source of POM to the Fraser River. Considering the ranges of lignin concentration in gymnosperm woods (61-152 mg/g OC) and non-woody tissues (26-68 mg/g OC, Appendix IV) and considering that the majority of the litterfall is composed by decaying leaves (needles) rather than woody debris (Duke et al., 1981; Heald, 1971), it appears from July 1999 samples that vascular plant matter comprises the large

majority of the OM input to the Fraser River. Therefore, as summer is likely to be the season of highest phytoplankton production, results imply that vascular plant tissues can potentially account for all of the OM in the river throughout the rest of the year. In the Columbia River, the %OC that is of terrestrial origin depends upon the season and related environmental conditions (including river flow, light availability, phytoplankton production). During periods of low autochthonous production such as late fall and winter (Figure 3.6 a, February 1998 and October 1997) or high flow conditions (May 1997) lignin content is high (16-35 and 11-30 mg/g OC respectively). However, contrary to the Fraser River and because of the angiosperm input to the Columbia River (higher lignin content than gymnosperm tissues, Appendix IV) and the low but present phytoplankton production, the contribution (as %OC) of terrestrially derived matter is lower than in the Fraser River.

Differences between the lignin content of Columbia River samples, the Fraser River and other North American Rivers could be solely explained if the sources contributing terrestrial OM to the Columbia River were somehow more lignin-rich than in the Fraser River (and the other North American Rivers). Three possible factors can individually contribute to such a difference of composition:

1. Different types of vascular plant tissues are contributing terrestrial OM. Numerous studies report differences in lignin content of plant tissues depending upon which type (A, a, G, g) they represent (Appendix IV and references therein). Fresh angiosperm tissues are typically more enriched in lignin than corresponding fresh gymnosperm tissues. This could possibly explain the difference between the Columbia and the Fraser River as the Columbia River OM is somewhat more enriched in angiosperm-derived tissues (see previous discussion). Although this explanation could be particularly relevant during periods of low autochthonous production, it cannot solely account for the differences observed recognizing that other North American Rivers are more enriched in angiosperm than the Columbia River (Figure 3.9).

2. Differences exist in the quality (i.e. level of degradation) of terrestrial OM. The literature indicates that lignin yield of vascular plant debris decreases significantly as aerobic degradation by white-rot fungi proceeds and that the extent of degradation can be reflected in the residual lignin composition by $(Ad/Al)_v$ ratios (Hedges et al., 1988). If the lignin component of Columbia River samples was significantly less degraded than that in other systems, this could help explain the observation. Unfortunately, the lignin degradation status of samples could not be evaluated since $(Ad/Al)_v$ measurements were compromised by collection on GFC filters (Appendix A).

3. SPM in Fraser River and twelve other North American Rivers is organic carbon-poor relative to that in the Columbia River [Figure 3.3 b)] and more concentrated on average. The presence in these rivers of a mineral-associated OM that is relict, not of immediate origin in the terrestrial ecosystem, and thus lignin-poor, could explain why their lignin content is lower (North American Rivers) or similar (Fraser River) to that of the Columbia River.

In the previous discussion, results from Columbia River samples collected in various years, seasons, tide and flow conditions were compared to snap-shot samples from the Fraser River collected along a downstream transect in July 1999. This type of approach could easily lead to erroneous conclusions. Indeed, close inspection of the dataset (Appendix V) reveals that the lignin content of SPM varies on a daily, monthly and seasonal basis. Two 12 h time series in October 1997 and February 1998 show lignin data spanning ranges close to that observed for the entire dataset (Figure 3.6b). The variations of lignin concentration are a direct response to tidal events (Appendix B), whose influence can be noticed as far as Bonneville Dam (RM145). A second point worth noting is the correlation between flow and lignin concentration. In May 1997 when the Columbia River discharge reached ~15,000 m^3s^{-1}, very low phytoplankton biomass was observed (Figure 3.6a) while lignin content (mg/gOC) was relatively high. On the other hand, flow in May 1992 was

only ~6,600 m^3s^{-1} and a strong phytoplankton response occurred resulting in low lignin content (dilution effect), half of that measured in May 1997. Although the period of concern is the same, the implications in terms of dominance of OM source to the river are opposite. As a consequence, comparing lignin data for these two cases with data obtained for the Fraser River in July 1999 would result in very different interpretations if flow dependency were not incorporated in the analysis. This example clearly shows that in order to be objectively compared, lignin results from different systems should originate from comparable conditions or these differences in conditions well described and taken into consideration upon interpretation.

The Columbia River is a complicated system where physical, chemical, geological and biological properties are strongly correlated. It is likely that the explanation for the higher than expected lignin results (compared to Fraser River and North American Rivers) is a combination of several of the features previously discussed, some of which can be seasonally dominant.

3.3 SUMMARY

1. SPM concentration in the Columbia River is low compared to other continental US rivers and shows no discernable seasonal trend. At any time, it appears dominated by fine particles (< 64 μm) and reveals only a slight direct dependency on flow.

2. The river POC content (wt%) ranges from 2.4 to 7.9% and displays a seasonal pattern resulting from dilution by mineral mass and flow variations.

3. (C/N)$_{at}$, Chla and n-FA data suggest that the river has a strong phytoplankton OM input that reaches its maximum during late spring and summer.

4. Compared to other US rivers (and Fraser River), the POM in the Columbia River exhibits high proportions of PN that is consistent with a strong phytoplankton contribution.

5. Lignin data do not exhibit seasonal trends as strongly as Chla and n-FA data, however they occasionally account for the complementarities between allochthonous and autochthonous matter as unique sources of OM to the river.

6. The Columbia River OM content appeared to be dominated by soil-derived matter during late fall and winter months, while spring and summer months exhibit an input dominated by phytoplankton production.

7. Differences in lignin sources among size fractions exist. Fine particles contain less lignin than coarse particles and show higher non-woody angiosperm tissue content. However, both fractions display a dominant woody gymnosperm origin.

8. The Fraser River contains higher SPM and much lower Chla/POC concentration than the Columbia River. It appears largely dominated by woody gymnosperm debris as opposed to the Columbia River that shows significant contribution from non-woody angiosperm. Observations are consistent with local vegetation coverage.

9. Lignin content of Columbia River resembles that of the Fraser and is higher than that of other US rivers east of the Rockies. Explanations include sources of terrestrial OM more lignin-rich or less decomposed. Also a mineral-associated relict OM introduced in the other rivers could explain this feature.

10. Daily, monthly, seasonal and interannual variation of lignin content at RM53 appears as the main feature of the Columbia River lignin story. Principal controls seem to be rainfall, runoff and river flow for a natural standpoint but human interferences (dams, irrigation, forest exploitation, etc) are growing bigger and could possibly dominate in the present days.

4: CONCLUSION

4.1 CONCLUSION

Traditionally, investigation of the OM characteristics of riverborne SPM involved the use of $(C/N)_{at}$ and Chla/POC measurements. The CuO oxidation provides another way to look both at allochthonous and autochthonous contributions. However, limitations of its use as a tracer of allochthonous matter are now apparent when samples are collected onto GFC filters.

During this work, I looked for POC changes in content and composition of the Columbia River SPM assuming that they were natural occurrences. Although not explicitly addressed here, intense human activities also are likely to be a determining factor. An interesting question concerns the Columbia River in the past and how human development modified its characteristics to those observed today. One hypothesis could be that the Columbia River resembled the Fraser River with high SPM yearlong preventing phytoplankton growth or greatly limiting it. The numerous dam constructions since the late 1960's (Dietrich, 1995; Harden, 1996) resulted in modifications of the flow patterns (see Figure 10 B, Sullivan et al., 2001). Although the annual mean discharge ($\sim$5,500 m^3s^{-1}) changed only slightly since 1878, changes in flow pattern happened including a decrease of seasonal flow variations, an increase of the minima in summer and fall (+ 1,000 m^3s^{-1}), and a reduction of the peak discharge (- 5,200 m^3s^{-1}) during spring freshet (Sherwood et al., 1990). The interconnected network of slack water reservoirs created by dams potentially induced changes in water quality characteristics. One of the possible changes is an enhancement of phytoplankton production (Sullivan et al., 2001). The overall result has possibly been to convert the Columbia River to a system capable of significant phytoplankton production. The river today shows a relatively low turbidity (SPM $\sim$10-30 mg/L) compared to the world's rivers (10 to > 10^3 mg/L; Berner and Berner,

1996). Therefore, assessing past and present characteristics of the river is of primary importance especially today when dams removal is discussed. The CuO oxidation technique may prove to be a valuable tool for help in making future policy decisions.

On a different note and to conclude this thesis, I would like to add that only by studying the past of the ecosystems in which we live, only by trying to understand the complicated physical, geological, chemical and biological processes that characterize these systems and by analyzing how human activity impacts them, can we build a good future by making wise and educated decisions. The CuO oxidation technique revealed itself as a promising tool for help in environmental management. Numerous features and applications of this technique have already been discovered and more are likely yet to come.

4.2 FUTURE RESEARCH

The present work constitutes a first use of the CuO oxidation technique to illuminate how POM in the Columbia River fits into the global carbon cycle. As during any research, many questions arose along the way, some of which were tested but many remain unanswered. Hereafter are suggestions for future research to improve the potential of the CuO oxidation technique and provide further insights about the lignin biogeochemistry of suspended particulate matter in the Columbia River.

4.2.1 About the CuO Technique

1. More tests on GFC filters should be conducted in order to:
   - Assess the chemical aspect of the recovery and degradation index problem.
   - Develop a method allowing the use GFC filters for lignin analysis (ex: removal of the sample from the filter by rinsing it off or degrading the filter).

2. Investigate the use of different type of filters to rapidly collect samples for lignin analysis by CuO oxidation.

3. Special attention should be paid to non-lignin phenols as this molecular information could bring interesting new and complementary insight to our understanding of SPM quality and factors leading to its variability.

4. Improvement of the CuO oxidation procedure are necessary to make it more suitable, costs efficient and easy to use. Especially:

   - Number of samples treated has to be increased from four to ten or twelve,

   - The overall procedure is too time-consuming (~ 2 days). Steps such as purging (overnight) could be reduced by decreasing the size of the purging box and increasing the pressure in the container, saving gas, time and money,

   - Improvement of the temperature control during the oxidation is suitable to assure a precise, constant and homogeneous temperature in the minibombs,

   The microwave technique proposed by Goni and Montgomery (2000) appears as a possible valuable alternative.

5. A thorough CuO analysis of the local vegetation and soil types should be done to provide future research with solid data to refer to.

6. Finally, a lignin database for samples of any type and origin (sediments, SPM, plant tissues, etc) could be created, accessible and updateable online. Data should include information about analyzing method, precision, type of sample, collection method/place/time and other useful data.

4.2.2 Concerning the Columbia River OM

Major questions about the seasonality and fate of terrestrial OM in the Columbia River remain unanswered. I would therefore organize future research around these two aspects.

4.2.2.1 Seasonality

1. Weekly SPM collection of size-fractionated and bulk samples (surface, middle and bottom) at RM53 would allow assessing monthly, seasonal and interannual changes in land-derived OM contribution and distribution among particles size.

2. Daily variability of SPM lignin content should be further investigated through 12 hours time series (1 entire tide). Bulk and size-fractionated surface, middle and bottom samples would show the influence of the tide on the quantity and quality of the OM found in the river.

4.2.2.2 Fate of terrestrial OM

1. Downstream study of river SPM and sediment samples throughout the Columbia basin, from tributaries into the coastal area (continental shelf) would give insights about the principal sources of terrestrial OM and the changes in quantity and quality it endures during its journey.

2. Special consideration should be paid to mudflats (i.e., sediments, waters leaving during ebb). Considering the large amount of land OM that does not reach coastal sediments, intense recycling or storage has to happen. ETM and mudflats might be key players in this processing.

3. The majority of the OC present in the river is in a dissolved form. Therefore, investigating DOC composition, sources and seasonality would seem valuable, particularly in context with POC.

4. Finally, on a paleo-geo-chemistry standpoint, it would be interesting to collect and analyze sediment core from the Columbia River for lignin analysis in order to investigate the past of the river.

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APPENDICES

Appendix I LMER-CRETM sampling locations
Samples were collected in the Columbia and the Fraser rivers (coordinates in degrees)

Site Name	Latitude N	Longitude W	Site Name	Latitude N	Longitude W
COLUMBIA RIVER					
Clatsop Spit	46.24650	123.98483	Rice Island	46.24800	123.68400
Astoria Dock	46.19333	123.98167	RM52	46.17400	123.20300
Hammond	46.20200	123.94100	RM53	46.18167	123.18317
Tansy Point	46.20167	123.94000	RM10-12	46.25100	124.04350
South Channel	46.20117	123.93850	Sand Island Outflux	46.26317	124.00000
North Channel	46.23500	123.89667	Sand Island Channel	46.26167	124.00166
Young's Bay Outflux	46.18583	123.88866			
Young's Bay	46.18333	123.88500	FRASER RIVER		
North Channel B	46.23500	123.88333	Steveston Bent, Buoy S19	49.11500	123.18000
Ebb ETM "Race"	46.23667	123.88330	Steveston Cut	49.11700	123.19100
Buoy 33	46.19667	123.86333	Crown Forest	49.10800	123.12800
Mid-Estuary, Buoy 37	46.19800	123.84700	Massey Tunnel	49.07800	123.07800
Mid-Estuary, Buoy 35B-37	46.19600	123.84067	Gravesend Reach	49.03300	123.03300
Mid-Estuary, Buoy 39	46.20100	123.81700	City Beach	49.96700	123.96700
East Mooring Basin	46.20833	123.79667	Westminister Quay	49.90500	123.90500
Cathlamet Bay	46.21033	123.79066	Queens Reach	49.83300	123.83300
Mid-Estuary, Tongue Point	46.21300	123.77000	Pattullo Bridge	49.21200	122.89500
Mid-Estuary, buoy 43	46.21600	123.74200	Douglas Island	49.81000	123.81000
Between Buoys 44 and 50	46.22200	123.73900	Sand Heads	49.10600	123.29300

Appendix II Reproducibility of GC-FID: utilization of Standard 4

Determination of the best method to be used for GC-FID: performance comparison between internal (ISTDM) and external (ESTDM) standard method. Std4: [BA] = 1.148 mg/mL, [t-cin] =1.240 mg/mL, [Et-Van] = 1.032 mg/mL, [FA] = 1.016 mg/mL

Solution content (ul) Std4 / Pyr / BSTFA	[t-Cin] inject	Area t-Cin	[Et-Van] inject	Area Et-Van ESTDM	[Et-Van] ISTDM	area Et-Van / area t-cin	[BA] inject	Area BA ESTDM	[BA] ISTDM	area BA / area t-cin	[FA] inject	Area FA ESTDM	[FA] ISTDM	area FA / area t-cin
Calibration lines														
50/0/50	1.24	180104	1.032	117409	0.808	0.652	1.148	170084	1.171	0.944	1.016	130662	0.900	0.725
25/75/100	0.31	51621	0.258	33307	0.200	0.645	0.287	49095	0.295	0.951	0.254	41786	0.251	0.809
50/50/100	0.62	103487	0.516	66309	0.397	0.641	0.574	89420	0.536	0.864	0.508	81761	0.490	0.790
75/25/100	0.93	141779	0.774	92040	0.604	0.649	0.861	125770	0.825	0.887	0.762	112188	0.736	0.791

Reproducibility of GC-FID: reruns (GC analysis) of the same sample (i.e. solution)

	[t-Cin] inject	Area t-Cin	[Et-Van] inject	Area Et-Van ESTDM	[Et-Van] ISTDM	area Et-Van / area t-cin	[BA] inject	Area BA ESTDM	[BA] ISTDM	area BA / area t-cin	[FA] inject	Area FA ESTDM	[FA] ISTDM	area FA / area t-cin
50/0/50	1.24	170988	1.032	110659	0.802	0.647	1.148	161510	1.171	0.945	1.016	135573	0.983	0.793
50/0/50	1.24	183698	1.032	118192	0.798	0.643	1.148	160286	1.082	0.873	1.016	145401	0.981	0.792
50/0/50	1.24	209032	1.032	133273	0.791	0.638	1.148	195458	1.159	0.935	1.016	163702	0.971	0.783
50/0/50	1.24	216423	1.032	137381	0.787	0.635	1.148	200869	1.151	0.928	1.016	168561	0.966	0.779
average		195035		124876	0.795	0.641		179531	1.141	0.920		153309	0.975	0.787
%Stdev		10.9%		10.1%	0.87%	0.87%		12.1%	3.52%	3.52%		10.1%	0.85%	0.86%

Appendix III CuO oxidation products of Lake Washington Standard Mud
Concentrations are in mg/g. Prahl et al., (1990) are unpublished data. /: data not available. %Reco: percentage recovery. c. var: coefficient of variation

		Van	AV	VA	SAld	AS	SA	CA	FA	V	S	C	Lig	S/V	C/V	%Vo	%So	Vad/al	Sad/al	%Reco
This study	average	0.37	0.13	0.17	0.15	0.05	0.05	0.05	0.04	0.67	0.26	0.09	1.02	0.39	0.13	19.0	21.5	0.46	0.36	64.1
n=6	c. var	5.0%	3.1%	7.7%	8.7%	12.2%	11.9%	22.6%	23.4%	5.4%	7.2%	14.8%	5.9%	4.3%	14.0%	5.6%	11.0%	4.8%	9.6%	
Other Publications																				
Requejo et al., (1986)	average	0.34	0.11	0.15	0.10	0.04	0.06	0.05	0.03	0.61	0.20	0.08	0.88	0.33	0.13	18.3	18.7	0.45	0.53	/
%OC=5.1%, n=3	c. var	0.9%	5.4%	7.8%	7.8%	5.5%	6.9%	28.0%	25.0%	/	/	/	4.2%	6.2%	24.0%	/	/	8.9%	9.4%	
Prahl et al., (1990)	average	0.32	0.11	0.12	0.13	0.06	0.05	0.06	0.04	0.71	0.29	0.12	1.13	0.41	0.17	18.9	24.3	0.43	0.39	78.5
%OC=5%, n=3	c. var	4.3%	9.3%	6.4%	8.0%	7.3%	8.6%	6.3%	6.6%	4.8%	5.2%	0.5%	2.5%	9.2%	5.9%	4.1%	7.3%	6.3%	16.5%	
Hedges and Ertel (1982)	average	0.27	0.09	0.11	0.12	0.04	0.04	0.04	0.04	0.47	0.20	0.09	0.76	0.43	0.19	18.5	21.4	0.40	0.29	/
%OC=5.05%	c. var	9.5%	9.8%	9.1%	12.9%	12.6%	15.3%	5.9%	12.1%	/	/	/	9.1%	4.7%	10.5%	/	/	2.5%	10.3%	

Appendix IV Lignin parameters for various vascular plant tissues
/: data not given; LIG: lignin concentration in mg/100mgOC

References	Sample origin	Part	LIG	S/V	C/V	Vadal	Sadal	V	S	C
								mg/100mgOC		
GYMNOSPERMS										
	woody tissue									
Hedges and Mann, 1979	Douglas fir (Pseudotsuga menziesii)	wood	6.10	0.00	0.00	/	/	6.10	0.00	0.00
Goni, 1992	Douglas fir (Pseudotsuga menziesii)	wood	15.18	0.01	0.01	0.23	0.31	14.94	0.11	0.13
This study (n = 3)	Douglas fir (Pseudotsuga menziesii)	wood	13.54	0.005	0.006	0.26	0.41	13.39	0.07	0.08
Goni and Thomas, 2000	Pine (Pinus taeda)	wood	14.20	0.07	0.02	0.19	0.11	13.10	0.86	0.26
Goni, 1992	Western red cedar (Thuja plicata)	wood	14.36	0.03	0.01	0.18	0.12	13.83	0.40	0.13
	Tsuga canadensis	wood	14.56	0.00	0.07	0.16	0.00	13.54	0.06	0.97
	Picea glauca	wood	13.78	0.00	0.01	0.19	0.00	13.66	0.01	0.12
	average		13.10	0.02	0.02	0.20	0.16	12.65	0.21	0.24
	non-woody tissue									
Hedges and Mann, 1979	Douglas fir (Pseudotsuga menziesii)	needle	3.42	0.06	0.57	/	/	2.10	0.12	1.20
Goni, 1992	Douglas fir (Pseudotsuga menziesii)	needle	3.24	0.07	0.36	0.35	0.51	2.26	0.17	0.81
	Western red cedar (Thuja plicata)	needle	2.55	0.02	0.23	0.29	1.75	2.04	0.04	0.47
Goni and Thomas, 2000	Pine (Pinus taeda)	needle	6.82	0.03	0.16	0.21	1.70	5.73	0.15	0.94
	average		4.01	0.05	0.33	0.28	1.32	3.03	0.12	0.85
ANGIOSPERMS										
	woody tissue									
Hedges and Parker, 1976	Silver maple (Acer saccharinum)	wood	24.50	2.80	0.00	/	/	6.50	18.00	0.00
	American holly (Ilex opaca)	wood	16.70	5.20	0.00	/	/	2.70	14.00	0.00
	Fir palm (Phoenix dactylifera)	wood	15.00	1.20	0.00	/	/	6.80	8.20	0.00
Hedges and Mann, 1979	Oregon oak (Castaena satina)	wood	20.00	1.50	0.00	/	/	8.00	12.00	0.00
	Red alder (Alnus rubra)	wood	10.80	2.00	0.00	/	/	3.60	7.20	0.00
Goni, 1992	Red alder (Alnus rubra)	wood	17.86	2.10	0.03	0.19	0.17	5.70	11.98	0.18
This study (n = 3)	Red alder (Alnus rubra)	wood	18.28	1.86	0.03	0.21	0.19	6.372	11.69	0.213
Goni, 1992	Acer saccharum	wood	24.86	3.13	0.01	0.11	0.09	6.01	18.81	0.04
	Oak (Quercus rubra)	wood	23.58	3.15	0.02	0.16	0.15	5.66	17.84	0.09
Hedges and Clark, 1986	Average for 16 trees (Amazon)	wood	19.30	1.54	0.01	0.14	0.14	7.57	11.66	0.08
	average		19.09	2.45	0.01	0.16	0.15	5.89	13.14	0.06

Appendix IV Continued
u: mathematically undefined due to division by zero.

References	Sample origin	Part	LIG	S/V	C/V	Vadal	Sadal	V	S	C
									mg/100mgOC	
ANGIOSPERMS										
	non-woody tissue									
Hedges and Parker. 1976	Black mangrove (Avicennia germinan	grass	4.20	1.20	1.10	/	/	1.60	0.70	0.87
	Jackbean (Canavalia maritima)	grass	2.84	2.00	1.10	/	/	1.30	1.50	1.40
	American holly (Ilex opaca)	leaves	4.48	2.60	0.44	/	/	0.70	1.40	0.74
	Cord grass (Spartina aterniflora)	grass	8.00	1.10	1.00	/	/	1.10	2.90	0.48
Hedges and Mann, 1979	Red alder (Alnus rubra)	leaves	3.17	0.44	0.54	/	/	3.00	1.90	3.10
	Oregon oak (Castaena satina)	leaves	4.18	1.20	0.36	/	/	1.60	2.00	0.58
Goni. 1992	Cord grass (Spartina aterniflora)	leaves	8.68	0.89	0.74	0.20	0.35	3.30	2.94	2.44
	Oak (Quercus rubra)	leaves	5.66	0.97	0.27	0.20	0.19	2.54	2.45	0.67
Goni and Thomas, 2000	Oak (Quercus virginiana)	leaves	4.79	1.50	0.42	0.34	0.37	1.63	2.48	0.68
	Juncus roemarianus	grass (dead)	14.60	1.70	1.10	0.48	0.37	3.82	6.63	4.16
	Spartina alterniflora	grass	6.98	1.10	1.10	0.22	0.41	2.23	2.39	2.37
Hedges and Clark. 1986	Average for 2 grasses (Amazon)	grass	9.10	0.77	1.31	0.22	0.21	2.28	3.87	9.10
	Average for 16 trees (Amazon)	leaves	3.66	0.93	0.19	0.17	0.16	1.61	0.33	3.66
	average		5.48	1.22	0.71	0.22	0.28	1.91	2.07	2.17
SOILS										
Prahl et al.. 1994	Litter. con. forest (Willam. Valley)		1.46	0.22	0.18	0.54	0.56	0.02	0.004	0.003
	Litter. con. forest (Willam. Valley)	> 250 um	3.56	0.72	0.28	0.39	0.37	0.07	0.01	0.02
	Litter. con. forest (Willam. Valley)	64-250 um	4.00	0.46	0.15	0.42	0.38	0.06	0.03	0.09
	Litter, con. forest (Willam. Valley)	< 64 um	3.26	0.34	0.12	0.49	0.48	0.04	0.01	0.04
Goni and Thomas, 2000	Oak and pine (shrubs)	top soil litter	11.30	0.72	0.40	0.23	0.23	5.33	3.85	2.14
	Oak and pine (shrubs)	bottom litter	10.40	0.57	0.31	0.37	0.36	5.52	3.16	1.72
OTHER										
Hedges and Mann. 1979	Eel grass (Zostera marina)	leaves	0.00	u	u	/	/	0.00	0.00	/
Hedges and Parker. 1976	Blue-green algea (Anacystis nidulans)	whole	0.00	u	u	/	/	0.00	0.00	0.00
	Mushroom (Lactarius deliciousus)	whole	0.00	u	u	/	/	0.00	0.00	0.00
	Brown algea (Sargassum sp.)	whole	0.00	u	u	/	/	0.00	0.00	0.00
Hedges and Clark. 1986	Average for 5 Macrophytes	whole	6.37	0.66	1.13	0.27	0.31	2.28	1.51	2.58

Appendix V Master table of samples data
CuO oxidation results. LIG: lignin content (mg/100mgOC). BE: Eversmeyer B.
unpublished data. / : data not available; * : data with low recovery. M, B, S: sample
collected at mid-water column, bottom and surface, respectively

Sample Name	Date	LIG	%Rec	S/V	C/V	%Vo	%So	Vadal	Sadal	S	V	C	3,5dBA /V
										mg/g dry sed			
RM53, bulk samples (GFC)													
92U1117 M	5/17/92	1.23	46	0.42	0.21	19.8	33.7	0.53	0.57	0.20	0.47	0.10	0.046
92U1125 M	5/17/92	0.69	42	0.65	0.16	21.4	36.5	0.78	0.82	0.18	0.27	0.04	0.048
92U1133 M	5/17/92	0.75	44	0.44	0.19	20.0	34.9	0.67	0.81	0.11	0.26	0.05	0.043
97aU1101 M	5/3/97	1.14	47	0.44	0.10	20.7	32.3	0.80	0.64	0.13	0.30	0.03	0.093
97aU1105 M	5/4/97	1.92	45	0.43	0.09	21.4	31.8	0.80	0.69	0.21	0.48	0.04	0.088
97aU2105 M*	5/15/97	3.02	20	0.39	0.10	25.2	41.7	1.57	1.92	0.25	0.62	0.06	0.088
97bU1105 M*	7/7/97	2.37	16	0.50	0.13	28.3	48.3	1.91	2.87	0.32	0.65	0.09	0.070
97cU1101 M	10/6/97	2.55	41	0.36	0.09	21.3	28.9	0.83	0.51	0.18	0.50	0.05	0.044
97cU1105 M	10/6/97	2.69	49	0.40	0.09	20.8	27.5	0.69	0.45	0.21	0.52	0.05	0.059
97cU1109 M	10/6/97	2.79	43	0.42	0.08	21.1	27.4	0.70	0.44	0.19	0.44	0.03	0.068
97cU1113 M	10/6/97	3.08	23	0.43	0.07	25.0	38.0	1.46	0.77	0.21	0.48	0.03	0.074
97cU1117 M	10/6/97	2.35	48	0.42	0.11	20.4	27.0	0.64	0.41	0.22	0.52	0.06	0.052
97cU1125 M	10/6/97	2.46	49	0.36	0.08	20.3	27.8	0.63	0.44	0.19	0.51	0.04	0.060
98aU1101 M	2/21/98	1.57	24	0.52	0.13	26.7	40.0	1.25	1.56	0.36	0.69	0.09	0.126
98aU1105 M	2/21/98	1.67	42	0.54	0.12	24.1	34.2	0.97	0.84	0.38	0.69	0.08	0.124
98aU1109 M	2/21/98	2.04	34	0.50	0.10	22.9	34.7	1.04	1.12	0.34	0.68	0.07	0.109
98aU1113 M*	2/22/98	2.99	18	0.53	0.11	22.5	31.6	0.94	0.63	0.35	0.65	0.07	0.092
98aU1117 M	2/21/98	3.50	21	0.56	0.19	25.3	37.5	1.91	1.44	0.54	0.98	0.19	0.121
98aU1121 M	2/21/98	2.74	35	0.46	0.09	24.0	35.1	0.96	1.07	0.31	0.66	0.06	0.121
98aU1125 M	2/22/98	1.93	43	0.52	0.11	23.0	33.0	0.87	0.80	0.27	0.52	0.06	0.069
99aU1105 M	6/16/99	2.17	25	0.45	0.13	26.1	39.7	1.10	0.97	0.27	0.60	0.08	0.051
99aU1117 M	6/17/99	2.39	31	0.47	0.12	23.8	37.1	0.81	0.65	0.26	0.56	0.07	0.057
99aU2117 M	6/22/99	2.29	24	0.43	0.10	26.2	42.5	1.02	1.01	0.28	0.66	0.07	0.031
RM53, bulk samples (GFC) from BE													
90U1113 M	9/21/90	0.73	/	0.33	0.07	19.0		0.24	0.22	0.12	0.36	0.03	0.051
92U1113 M	5/17/92	0.73	/	0.34	0.10	17.6		0.28	0.26	0.12	0.37	0.04	0.051
92U1121 M	5/17/92	0.92	/	0.35	0.12	18.5		0.27	0.19	0.14	0.39	0.05	0.059
RM53, ultrafiltration samples													
96U1105M<64	7/18/96	0.94	64	0.44	0.11	19.4	33.8	0.61	0.45	0.11	0.26	0.03	0.110
97aU1121M<64	5/4/97	1.54	42	0.49	0.11	20.5	29.8	0.88	0.59	0.13	0.26	0.03	0.141
97aU2105M<64	5/15/97	1.13	74	0.43	0.09	17.5	28.6	0.48	0.31	0.07	0.16	0.01	0.058
97cU1109M<64	10/6/97	1.92	45	0.35	0.07	25.5	26.1	0.81	0.31	0.07	0.20	0.02	0.084
99aU1109M<64	6/16/99	0.99	68	0.35	0.07	18.1	33.2	0.46	0.48	0.09	0.25	0.02	0.095
99aU2105M<64	6/22/99	0.99	62	0.48	0.10	19.0	29.0	0.55	0.36	0.10	0.21	0.02	0.097
97aU1121M>64	5/4/97	5.54	41	0.19	0.07	23.5	16.9	0.74	0.23	0.14	0.75	0.05	0.062
97aU2105M>64	5/15/97	4.37	77	0.34	0.06	17.0	24.2	0.29	0.20	0.18	0.55	0.03	0.027
97cU1109M>64	10/6/97	5.47	35	0.18	0.06	26.5	19.8	0.86	0.30	0.16	0.88	0.06	0.042
99aU1109M>64	6/16/99	2.16	65	0.34	0.09	16.9	25.6	0.40	0.35	0.56	1.67	0.15	0.048
99aU2105M>64	6/22/99	1.49	64	0.17	0.06	19.6	59.0	0.69	0.48	0.24	1.43	0.09	0.063

Appendix V Continued

Sample Name	C4DA	C4:1DA	Pg	C14FA	C16:1FA	C16FA	POC wt%	SPM mg/L	C/N at	Chla mg/gOC	Al wt%
			mg/gOC								
RM53, bulk samples (GFC)											
92U1117 M	1.66	5.85	3.47	9.89	8.61	1.55	6.3	20.4	7.1	58.5	/
92U1125 M	3.29	6.18	2.87	13.96	12.42	2.03	7.1	12.6	6.5	46.4	5.31
92U1133 M	1.99	5.15	3.25	8.87	8.03	1.11	5.6	24.1	7.9	40.1	5.92
97aU1101 M	2.55	3.65	1.24	1.81	1.03	0.38	4.0	25.8	11.1	5.2	7.90
97aU1105 M	3.72	7.13	1.94	2.25	1.28	0.37	3.8	31.6	10.9	7.5	6.38
97aU2105 M*	6.88	13.07	1.17	3.20	1.86	0.69	3.1	34.8	9.1	10.6	/
97bU1105 M*	8.08	17.44	1.66	8.90	8.59	2.06	4.5	27.1	9.0	22.0	7.56
97cU1101 M	2.83	4.52	1.97	1.29	1.14	0.48	2.8	36.0	10.9	6.7	6.61
97cU1105 M	3.78	5.32	2.41	2.47	2.62	0.87	2.9	28.7	10.8	9.9	7.14
97cU1109 M	5.16	7.17	2.85	2.88	3.06	0.87	2.4	25.2	9.7	12.9	8.44
97cU1113 M	13.97	17.56	2.90	7.40	8.59	2.88	2.4	17.2	8.5	16.1	8.35
97cU1117 M	4.03	5.11	2.77	2.36	2.65	0.98	3.4	18.8	9.7	10.5	8.29
97cU1125 M	3.57	5.81	2.51	1.58	1.65	0.56	3.0	26.4	10.4	9.8	7.35
98aU1101 M	6.35	5.03	1.31	4.37	2.59	1.15	7.2	10.9	10.0	12.5	8.71
98aU1105 M	3.68	5.40	1.43	4.24	3.12	0.89	6.9	10.2	10.5	13.1	8.59
98aU1109 M	5.60	8.11	1.88	3.22	2.04	0.62	5.4	11.9	10.3	11.1	8.52
98aU1113 M*	6.54	11.36	5.65	4.13	3.28	1.16	3.6	15.3	10.3	10.8	8.88
98aU1117 M	4.45	5.73	2.55	8.08	5.71	1.55	4.9	14.4	10.5	11.1	8.4
98aU1121 M	6.84	10.30	1.80	4.93	3.37	1.07	3.8	15.1	10.5	10.8	8.32
98aU1125 M	5.07	5.87	1.51	3.62	2.57	0.63	4.4	14.1	10.4	10.7	8.48
99aU1105 M	6.38	13.49	2.50	9.00	7.57	2.54	4.4	15.9	8.4	29.0	/
99aU1117 M	5.05	13.89	2.02	8.47	5.70	1.05	3.7	18.8	8.4	23.2	/
99aU2117 M	5.63	21.57	2.52	10.50	9.61	1.84	4.4	17.1	9.3	31.4	7.17
RM53, bulk samples (GFC) from BE											
90U1113 M	0.89	7.55	1.94	4.03	3.01	2.06	7.0	10.8	8.6	13.0	/
92U1113 M	1.40	7.64	2.83	5.59	3.66	1.20	7.2	21.1	7.7	29.7	/
92U1121 M	2.59	10.13	3.81	7.41	2.72	1.40	6.3	15.6	6.4	51.0	/
RM53, ultrafiltration samples											
96U1105M<64	2.62	3.87	5.18	3.17	2.07	0.43	4.3	11.6	11.1		/
97aU1121M<64	4.49	5.58	3.49	4.71	4.69	1.16	2.7	24.4	14.7		/
97aU2105M<64	3.06	3.09	2.47	3.88	3.64	1.00	2.1	27.0	13.7	/	/
97cU1109M<64	7.35	4.19	3.14	5.11	5.51	2.24	1.5	19.9	14.4	/	/
99aU1109M<64	2.51	3.79	4.18	3.36	2.51	0.73	3.6	10.0	10.1	/	/
99aU2105M<64	2.90	3.22	3.58	3.91	2.75	0.81	3.3	12.2	9.5	/	/
97aU1121 M>64	16.19	4.60	2.68	9.54	3.55	6.65	1.7	4.7	25.3		/
97aU2105M>64	3.43	3.07	2.62	4.53	2.55	1.01	1.7	11.0	20.3		/
97cU1109M>64	14.65	4.48	2.01	7.44	6.69	5.22	2.0	3.4	25.2	/	/
99aU1109M>64	3.15	3.99	1.65	5.55	2.79	1.09	11.0	1.5	16.9	/	/
99aU2105M>64	1.16	1.90	6.15	12.39	6.01	1.41	11.8	1.8	9.8	/	/

Appendix V Continued

Sample Name	Date	LIG	%Rec	S/V	C/V	%Vo	%So	Vadal	Sadal	S	V	C	dBA/V
										mg/g dry sed			
Fraser River, transect, bulk samples (GFC)													
99bT02 B	7/16/99	1.75	59	0.25	0.07	19.2	15.7	0.49	0.43	0.03	0.11	0.01	0.077
99bT03 B	7/9/99	3.60	62	0.27	0.06	19.5	19.7	0.44	0.31	0.08	0.30	0.02	0.059
99bT04 B	7/16/99	1.65	57	0.34	0.11	19.5	22.8	0.56	0.45	0.07	0.20	0.02	0.078
99bT05 B	7/16/99	2.01	66	0.30	0.07	18.7	21.2	0.41	0.30	0.08	0.28	0.02	0.069
99bT06 B	7/16/99	1.87	56	0.32	0.10	20.6	24.9	0.57	0.48	0.06	0.20	0.02	0.088
99bT07 B	7/16/99	2.44	52	0.28	0.07	19.7	22.6	0.49	0.34	0.07	0.25	0.02	0.056
99bT08 B	7/18/99	2.09	62	0.33	0.07	19.8	23.8	0.58	0.48	0.05	0.15	0.01	0.098
99bT09 B	7/18/99	2.00	62	0.36	0.06	19.9	23.6	0.56	0.37	0.05	0.14	0.01	0.099
99bT10 B	7/18/99	2.28	44	0.36	0.07	21.3	24.7	0.71	0.53	0.07	0.18	0.01	0.106
Fraser River, bulk samples (GFC)													
99bL1149 S	7/9/99	2.12	69	0.29	0.07	18.3	19.8	0.38	0.30	0.06	0.22	0.02	0.056
99bL1149 B	7/9/99	1.71	63	0.27	0.06	18.8	22.4	0.43	0.36	0.06	0.24	0.01	0.058
99bL1153 B	7/9/99	1.78	62	0.31	0.08	19.7	21.5	0.43	0.33	0.07	0.22	0.02	0.062
Fraser River, ultrafiltration samples													
99bL1268AB>64	7/14/99	7.12	76	0.32	0.06	16.2	18.8	0.35	0.28	0.49	1.51	0.09	0.037
99bL1268AB<64	7/14/99	2.54	63	0.19	0.03	18.5	26.2	0.44	0.09	0.04	0.22	0.01	0.030
Estuary, North channel, bulk samples (GFC)													
97aF1114 B	5/6/97	4.98	40	0.44	0.21	20.0	28.9	0.82	0.56	0.40	0.91	0.19	0.054
97bF1208 B	7/16/97	1.95	22	0.43	0.26	18.8	37.4	1.33	0.91	0.13	0.30	0.08	0.055
97bN1113 B	7/11/97	5.22	80	0.41	0.15	21.0	36.3	0.70	0.47	0.12	0.28	0.04	0.062
97cF1305 B	10/14/97	1.38	57	0.40	0.09	19.4	25.0	0.48	0.32	0.14	0.36	0.03	0.050
98aF1209 B	2/23/98	2.51	35	0.37	0.08	20.5	26.2	0.72	0.44	0.33	0.90	0.08	0.024
Estuary, South channel, bulk samples (GFC)													
97aE1206 B	5/7/97	1.82	44	0.40	0.14	19.5	28.6	0.66	0.51	0.14	0.34	0.05	0.062
97aF1205 B	5/7/97	0.60	68	0.44	0.10	17.6	25.1	0.52	0.35	0.12	0.28	0.03	0.083
97bE1109 B	7/9/97	1.34	52	0.42	0.11	18.2	25.8	0.48	0.39	0.17	0.41	0.04	0.056
97cE1307 B	10/15/97	1.57	43	0.39	0.09	20.2	27.2	0.58	0.37	0.17	0.43	0.04	0.042
97cF1117 B	10/14/97	3.29	46	0.37	0.07	20.4	26.7	0.54	0.37	0.34	0.91	0.07	0.035

Appendix V Continued

Sample Name	C4DA	C4:1DA	Pg	C14FA	C16:1FA	C16FA	POC wt%	SPM mg/L	C/N at	Chla mg/gOC	Al wt%
			mg/gOC								
Fraser River, transect, bulk samples (GFC)											
99bT02 B	1.56	2.47	1.13	0.68	0.56	0.23	0.80	126.0	21.2	1.7	/
99bT03 B	2.82	5.21	2.09	0.77	1.19	0.57	1.11	102.1	23.5	1.3	/
99bT04 B	2.22	2.07	0.90	1.20	1.02	0.88	1.71	63.7	21.8	1.3	/
99bT05 B	2.30	2.06	1.15	0.89	0.96	0.60	1.92	67.4	23.2	1.1	/
99bT06 B	3.20	2.43	0.74	1.28	1.23	1.38	1.53	66.3	22.8	1.5	/
99bT07 B	3.43	2.90	0.94	0.90	0.83	0.61	1.36	81.6	26.0	1.2	/
99bT08 B	2.69	2.74	0.88	0.92	0.99	0.83	0.98	149.2	29.1	1.0	/
99bT09 B	6.75	5.13	1.57	2.67	2.62	2.89	0.99	118.6	40.6	1.2	/
99bT10 B	2.77	4.13	1.14	0.97	1.03	0.56	1.16	174.7	23.6	0.8	/
Fraser River, bulk samples (GFC)											
99bL1149 S	1.69	2.59	1.04	0.78	0.95	0.41	1.39	79.6	21.4	1.5	/
99bL1149 B	1.76	1.93	0.82	0.59	0.71	0.48	1.84	68.5	21.0	1.4	/
99bL1153 B	1.52	2.29	0.90	0.84	0.98	0.57	1.69	68.7	19.3	1.3	/
Fraser River, ultrafiltration samples											
99bL1268AB>64	2.20	2.36	0.32	0.53	0.59	0.46	2.93	144.7	28.4	/	/
99bL1268AB<64	1.89	4.04	0.00	2.06	1.12	2.66	1.06	591.1	25.2	/	/
Estuary, North channel, bulk samples (GFC)											
97aF1114 B	3.87	10.74	5.04	2.72	1.90	0.45	3.0	96.0	12.2	3.5	/
97bF1208 B	2.35	10.31	5.91	2.05	1.82	0.43	2.6	289.2	7.3	14.5	/
97bN1113 B	5.69	19.87	14.95	7.60	5.50	1.37	0.8	88.2	8.5	34.5	/
97cF1305 B	1.02	1.83	0.89	0.57	0.38	0.18	3.9	337.5	12.9	1.2	/
98aF1209 B	1.40	5.23	2.68	0.49	0.40	0.08	5.2	145.4	13.4	3.4	/
Estuary, South channel, bulk samples (GFC)											
97aE1206 B	1.22	3.31	1.07	0.88	0.64	0.16	2.9	172.0	8.7	2.1	/
97aF1205 B	0.51	0.80	0.52	0.40	0.23	0.07	7.2	108.4	/	0.8	/
97bE1109 B	0.42	0.48	1.41	2.43	0.41	0.09	4.7	15.2	9.0	6.4	/
97cE1307 B	0.26	0.45	0.86	0.83	0.08	0.04	4.0	32.8	13.5	2.5	/
97cF1117 B	2.11	5.27	2.03	0.97	0.59	0.26	4.0	75.8	13.6	3.0	/

Appendix V Continued

Sample Name	Date	LIG	%Rec	S/V	C/V	%Vo	%So	Vadal	Sadal	S	V	C	dBA/V
										mg/g dry sed			
Estuary, North channel, ultrafiltration samples													
97aF1114B<64	5/6/97	6.28	60	0.43	0.12	17.5	20.9	0.31	0.22	0.32	0.73	0.09	0.034
97bE2302B<64	7/16/97	1.74	54	0.43	0.08	18.8	29.8	0.54	0.30	0.11	0.26	0.02	0.072
97bF1208B<64	7/16/97	1.62	64	0.41	0.09	18.5	29.9	0.55	0.38	0.10	0.25	0.02	0.076
97cF1305B<64	10/14/97	2.99	61	0.42	0.09	18.9	23.1	0.53	0.28	0.17	0.40	0.03	0.071
98aE1207B<64	2/23/98	2.21	43	0.38	0.07	18.2	24.3	0.47	0.33	0.14	0.37	0.03	0.062
98aF1209B<64	2/23/98	3.46	17	0.53	0.14	19.5	22.8	0.88	0.31	0.25	0.48	0.07	0.078
99aL11103B<64	6/19/99	1.78	63	0.38	0.09	17.9	27.4	0.51	0.34	0.09	0.24	0.02	0.075
99aL21127B<64	6/27/99	2.19	50	0.42	0.14	17.6	25.5	0.59	0.41	0.11	0.27	0.04	0.090
99aL21177B<64	6/28/99	2.54	48	0.38	0.09	21.2	30.3	0.75	0.56	0.13	0.34	0.03	0.099
99aL21227B<64	6/27/99	2.27	51	0.41	0.10	18.0	28.2	0.58	0.40	0.12	0.29	0.03	0.092
97aF1114B>64	5/6/97	0.87	58	0.49	0.13	18.3	25.3	0.74	0.36	0.09	0.19	0.02	0.090
97bE2302B>64	7/16/97	1.90	59	0.42	0.16	18.8	26.7	0.35	0.31	0.45	1.09	0.17	0.039
97bF1208B>64	7/16/97	3.05	68	0.41	0.09	17.5	26.2	0.38	0.29	0.30	0.73	0.06	0.042
97bN1113B>64	7/11/97	1.17	53	0.45	0.22	18.7	31.8	0.47	0.35	0.46	1.01	0.22	0.044
97cF1305B>64	10/14/97	5.59	71	0.37	0.08	16.7	20.7	0.33	0.26	0.62	1.69	0.14	0.034
98aE1207B>64	2/23/98	4.59	77	0.26	0.04	16.5	18.8	0.34	0.19	0.08	0.32	0.01	0.025
98aF1209B>64	2/23/98	6.04	72	0.38	0.09	16.0	18.9	0.28	0.21	0.89	2.34	0.22	0.024
99aL11103B>64	6/19/99	3.80	65	0.35	0.08	18.6	21.4	0.52	0.24	0.07	0.20	0.02	0.032
99aL21127B>64	6/27/99	5.17	59	0.37	0.07	19.1	22.5	0.37	0.23	0.42	1.13	0.08	0.032
99aL21177B>64	6/28/99	6.01	69	0.33	0.07	17.4	22.1	0.36	0.30	0.27	0.80	0.06	0.040
99aL21227B>64	6/27/99	5.58	47	0.38	0.09	18.0	21.5	0.50	0.30	0.13	0.34	0.03	0.046
Estuary, South channel, ultrafiltration samples													
97aE1206B<64	5/7/97	2.18	63	0.40	0.11	19.2	24.9	0.53	0.34	0.13	0.32	0.04	0.072
97aF1205B<64	5/7/97	2.26	74	0.36	0.09	18.7	26.7	0.49	0.31	0.08	0.23	0.02	0.067
97bE1109B<64	7/9/97	1.49	62	0.40	0.09	18.9	32.9	0.56	0.39	0.10	0.24	0.02	0.081
97bE2104B<64	7/15/97	1.22	68	0.42	0.08	17.4	32.0	0.52	0.32	0.08	0.20	0.02	0.062
97cE1307B<64	10/15/97	2.48	61	0.38	0.09	18.7	25.0	0.48	0.27	0.13	0.34	0.03	0.064
97cF1117B<64	10/14/97	2.54	62	0.39	0.10	18.0	23.6	0.52	0.30	0.13	0.34	0.04	0.066
98aE1304B<64	2/28/98	2.61	66	0.41	0.10	18.3	24.1	0.54	0.30	0.16	0.38	0.04	0.068
97aE1206B>64	5/7/97	2.74	56	0.40	0.12	16.8	21.6	0.30	0.26	0.56	1.38	0.16	0.035
97aF1205B>64	5/7/97	5.45	72	0.36	0.08	17.0	20.7	0.30	0.22	0.27	0.75	0.06	0.030
97bE1109B>64*	7/9/97	3.44	37	0.09	0.05	25.4	/	0.98	/	0.24	2.78	0.14	0.028
97bE2104B>64	7/15/97	0.82	49	0.44	0.25	19.8	34.6	0.45	0.31	0.39	0.87	0.22	0.055
97cE1307B>64	10/15/97	4.67	74	0.32	0.08	19.7	18.3	0.82	0.22	0.04	0.13	0.01	0.035
97cF1117B>64	10/14/97	5.43	69	0.44	0.13	16.1	20.4	0.29	0.23	1.93	4.34	0.58	0.024
98aE1304B>64	2/28/98	5.12	72	0.36	0.08	16.4	20.4	0.29	0.21	0.26	0.71	0.06	0.025

Appendix V End

Sample Name	C4DA	C4:1DA	Pg	C14FA	C16:1FA	C16FA	POC wt%	SPM mg/L	C/N at	Chla mg/gOC	Al wt%
				mg/gOC							
Estuary, North channel, ultrafiltration samples											
97aF1114 B<64	4.27	4.59	5.09	4.99	2.94	1.22	1.8	47.4	15.5	/	7.9
97bE2302 B<64	3.81	4.08	2.55	4.41	3.31	1.18	2.3	137.1	13.5	/	8.0
97bF1208 B<64	4.09	4.67	2.53	3.43	2.84	0.48	2.3	188.3	13.3	/	7.8
97cF1305 B<64	4.00	3.99	1.88	1.26	0.93	0.63	2.0	437.3	16.3	/	8.2
98aE1207 B<64	2.67	2.96	1.95	2.72	1.43	0.63	2.4	41.0	15.8	/	/
98aF1209 B<64	4.62	3.85	2.04	2.58	2.16	1.52	2.3	209.4	17.1	/	/
99aL11103 B<64	3.47	3.42	2.33	4.51	2.52	1.28	2.0	52.4	11.8	/	/
99aL21127 B<64	1.66	4.21	2.79	7.48	4.60	2.67	1.9	201.7	12.4	/	/
99aL21177 B<64	5.34	6.55	4.12	4.32	3.88	1.03	2.0	342.0	11.5	/	/
99aL21227 B<64	5.35	5.29	3.31	4.95	3.64	1.59	1.9	141.1	11.9	/	/
97aF1114 B>64	2.24	1.53	0.93	1.39	1.05	0.40	3.5	9.3	12.9	/	8.2
97bE2302 B>64	1.97	3.89	11.46	1.95	2.43	1.50	9.0	24.6	8.4	/	6.6
97bF1208 B>64	2.94	3.97	3.96	3.62	3.77	0.76	3.6	63.6	14.3	/	7.3
97bN1113 B>64	1.96	3.89	9.19	1.34	1.34	0.66	14.5	9.9	8.6	/	5.5
97cF1305 B>64	1.87	2.58	2.95	0.72	0.65	0.26	4.4	97.0	18.8	/	8.1
98aE1207 B>64	4.62	1.00	0.63	4.82	1.38	1.82	0.9	34.7	24.9	/	/
98aF1209 B>64	1.27	2.02	1.61	0.53	0.55	0.18	5.7	25.6	26.0	/	/
99aL11103 B>64	6.06	1.86	1.57	5.05	3.77	2.52	0.8	82.2	24.6	/	/
99aL21127 B>64	3.81	2.67	1.57	5.94	4.37	5.68	3.2	77.2	15.8	/	/
99aL21177 B>64	3.81	3.42	2.76	2.85	2.20	0.71	1.9	224.8	18.2	/	/
99aL21227 B>64	3.81	2.67	1.57	5.94	4.37	5.68	0.9	205.5	32.6	/	/
Estuary, South channel, ultrafiltration samples											
97aE1206 B<64	3.11	2.89	1.92	1.89	1.37	0.54	2.2	176.4	15.5		9.29
97aF1205 B<64	4.45	3.16	1.37	1.53	1.02	1.01	1.5	256.6	17.3		8.16
97bE1109 B<64	3.77	4.32	2.84	3.32	2.63	0.63	2.4	9.7	14.2	/	/
97bE2104 B<64	3.19	3.13	1.92	4.61	2.43	0.90	2.4	34.9	14.0	/	7.13
97cE1307 B<64	3.65	3.41	1.89	1.70	0.99	0.85	2.0	46.2	16.1	/	/
97cF1117 B<64	3.46	2.94	1.52	1.36	1.23	0.58	2.0	89.2	16.2	/	8.17
98aE1304 B<64	3.21	3.05	1.35	1.49	0.81	0.54	2.2	126.0	17.0	/	/
97aE1206 B>64	1.74	3.30	4.76	1.70	1.26	0.40	7.7	36.1	10.2		7.65
97aF1205 B>64	2.83	0.25	0.93	0.77	0.44	0.64	2.0	166.9	29.8		8.09
97bE1109 B>64*	14.94	2.71	0.31	10.49	5.06	6.17	9.2	0.6	15.0	/	/
97bE2104 B>64	1.77	3.67	8.83	1.61	1.36	1.45	18.1	2.8	8.7	/	4.51
97cE1307 B>64	11.79	0.64	0.70	4.37	0.99	6.75	0.4	101.3	36.3	/	/
97cF1117 B>64	0.85	1.97	3.43	0.44	0.46	0.14	12.6	3.8	20.0	/	6.54
98aE1304 B>64	2.75	1.88	0.80	0.94	0.50	0.65	2.0	50.5	26.4	/	/

APPENDIX A

ADVANCEMENT OF THE CuO OXIDATION TECHNIQUE

During this study, a considerable amount of effort was expended to understand, apply and improve the CuO oxidation technique. These efforts eventually yielded a detailed evaluation of the strengths and weaknesses of the CuO oxidation technique, presented hereafter.

$(Ad/Al)_v$ AS A VALUABLE DEGRADATION INDICATOR

Hedges et al. (1988) showed that the ratio of vanillic acid to vanillin $[(Ad/Al)_v]$ could be used as a reliable indicator of the decomposition state of vascular plant OM in sedimentary samples. They experimentally monitored in the laboratory the decomposition of Birchwood by white rot fungi, the main lignin decomposer on land, and clearly showed that $(Ad/Al)_v$ increased with decomposition time. Later studies with white and soft-rot fungi (Hedges et al., 1988 b; Nelson et al., 1995; Goni et al., 1993) confirmed this feature, which does not occur under brown-rot degradation action (Crawford, 1981, Hedges et al., 1988 b). However, some fresh non-woody angiosperm and gymnosperm tissues contain large quantity of phenolic acids including vanillic acid ester-bound to polysaccharides (Whitehead et al., 1981; Atsushi et al., 1984; Hartley and Ford, 1989; Yamamoto et al., 1989). Although fresh, the CuO oxidation of these tissues yields elevated $(Ad/Al)_v$ ratios (0.3-0.5) (Benner et al., 1990; Goni, 1992; Goni and Hedges, 1992; Goni et al., 1993), thereby potentially complicating the use of this parameter as a diagenetic index. None of these studies reported the percentage recovery (%Reco) obtained for the samples analyzed. This issue is important given that one major result from my work is that $(Ad/Al)_v$ is sensitive to the efficiency of sample recovery with which it inversely correlates.

While no indication of such a feature was observed in preliminary testing with solids, this artifact became apparent when river samples on GFC were analyzed. Indeed, a first obvious observation from Figures A1 a), b) was that $(Ad/Al)_v$ was inversely correlated to %Reco, that is the ratio went up as %Reco went down (r^2 = 0.87). This feature seemed independent of sample grain-size and amount analyzed as it was observed for both size-fractionated (> 64 µm and < 64 µm) and bulk samples and for a range of sample weights. However, worse recoveries were achieved for bulk samples on GFC filters. The phenomenon occurred in samples from the Fraser River, the Columbia River and estuary, and Lake Washington [Figure A1 c)] but not necessarily to the same extend. For example, bulk and size-fractionated samples from the Fraser River and LWSM resulted in higher %Reco than those from the Columbia River. A correlation with the SPM quality seemed possible. An important aspect was that this decreasing trend was strongly associated with the sample type. Indeed, as Figures A1 a) and b) show, lower %Reco ($\leq$ 50%) and higher $(Ad/Al)_v$ ratio were obtained for the Columbia River GFC samples compared to homogenized size-fractionated samples for which %Reco usually ranged from 50% to 80%. An analysis of bulk solid samples (not available) would have allowed to dissociate the effect of the filter from that, possibly, that of the particles size and SPM quality. From a laboratory standpoint, emulsions were frequently noticed during the extraction phase in the separatory funnels containing samples laid down on GFC but not on solid samples without a GFC support.

The recovery aspect of the procedure happened after the CuO oxidation phase, when a solution of Et-Van was introduced into the reaction mixture. Therefore, the phenomenon accountable for the alteration of the %Reco and $(Ad/Al)_v$ had to take place during the workup phase. However, the conditions leading to the problem could stem from the chemistry of the solution provided by CuO oxidation reaction. In order to illuminate the cause for the recovery-$(Ad/Al)_v$ artifact, two experiments were conducted.
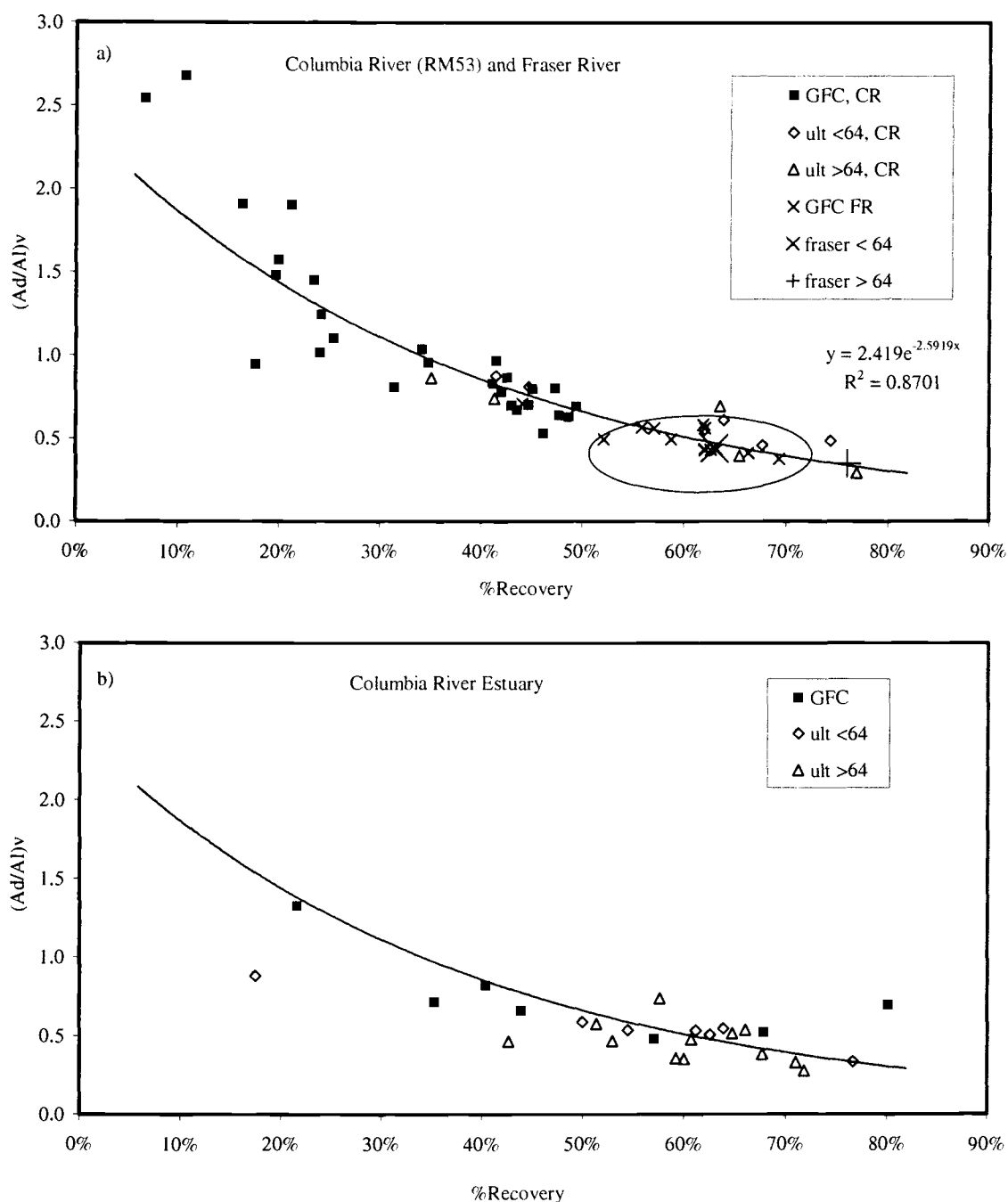
Figure A1 Correlation between $(Ad/Al)_v$ and sample's %Recovery
CR: Columbia River; FR: Fraser river transect; ult: size-fractionated homogenized
solids; GFC: coarse glass fiber filter sample. Trend is for all the data of plot a).
Decreasing trend in plot b) is that of plot a). Note: circled data in plot a) originated
from the Fraser River and seemed less influenced by GFC filters than those from the
Columbia River

In the first experiment, the possibility that GFC filters were responsible for lower %Reco and higher (Ad/Al)$_v$ values was investigated by oxidizing four homogenized solid samples and LWSM samples in minibombs containing a glass fiber filter (tabulated data in Tables A1 and A2). The GFC filter was added to "recreate" the conditions of an actual solid sample collected on a GFC. Results shown in Figures A1 c) and d) fit the trend previously described and confirmed that GFC filter artificially increased (Ad/Al)$_v$ and decreased %Reco. Although leading to lower %Reco and higher (Ad/Al)$_v$ results than their GFC-free counterparts, these samples did not display however, recovery values as low as those obtained with bulk GFC samples. This possibly implied that the presence of the GFC filters was indeed corrupting the results, but also possibly that SPM quality might worsen the problem. It is also possible that the quantity of Si(OH)$_4$ (from the filter) present during the entire CuO oxidation process was important. While no more GFC filters remained that were identical to those used for sample collection, this experiment was conducted with slightly smaller filters (50 mm GFF, Whatman). This fact could solely explain the "better" results obtained than those from real samples, as smaller filters would result in smaller matrix effects. Finally, a crucial result from this first test was that while the decomposition tracer was corrupted by the use of the GFC filters, none of the other lignin or non-lignin (i.e., n-FA content) parameters appeared affected (Figures A.2).

In the second experiment, blank samples from the same oxidation batch were compared, some containing only the reactants necessary for the CuO oxidation, the others containing a GFC filter as well. Instead of introducing solely Et-Van after the oxidation, a known amount of Std16 was used. The dual objective was to (1) provide further proof of %Reco decrease and (Ad/Al)$_v$ increase due to GFC filters and (2) determine how %Reco varied between various compounds. Results shown in Figure A3 are striking. First, GFC blank recoveries (40%-65%, mean: 61%) were lower than those of blanks without filters (40%-90%, mean: 73%) for the majority of the
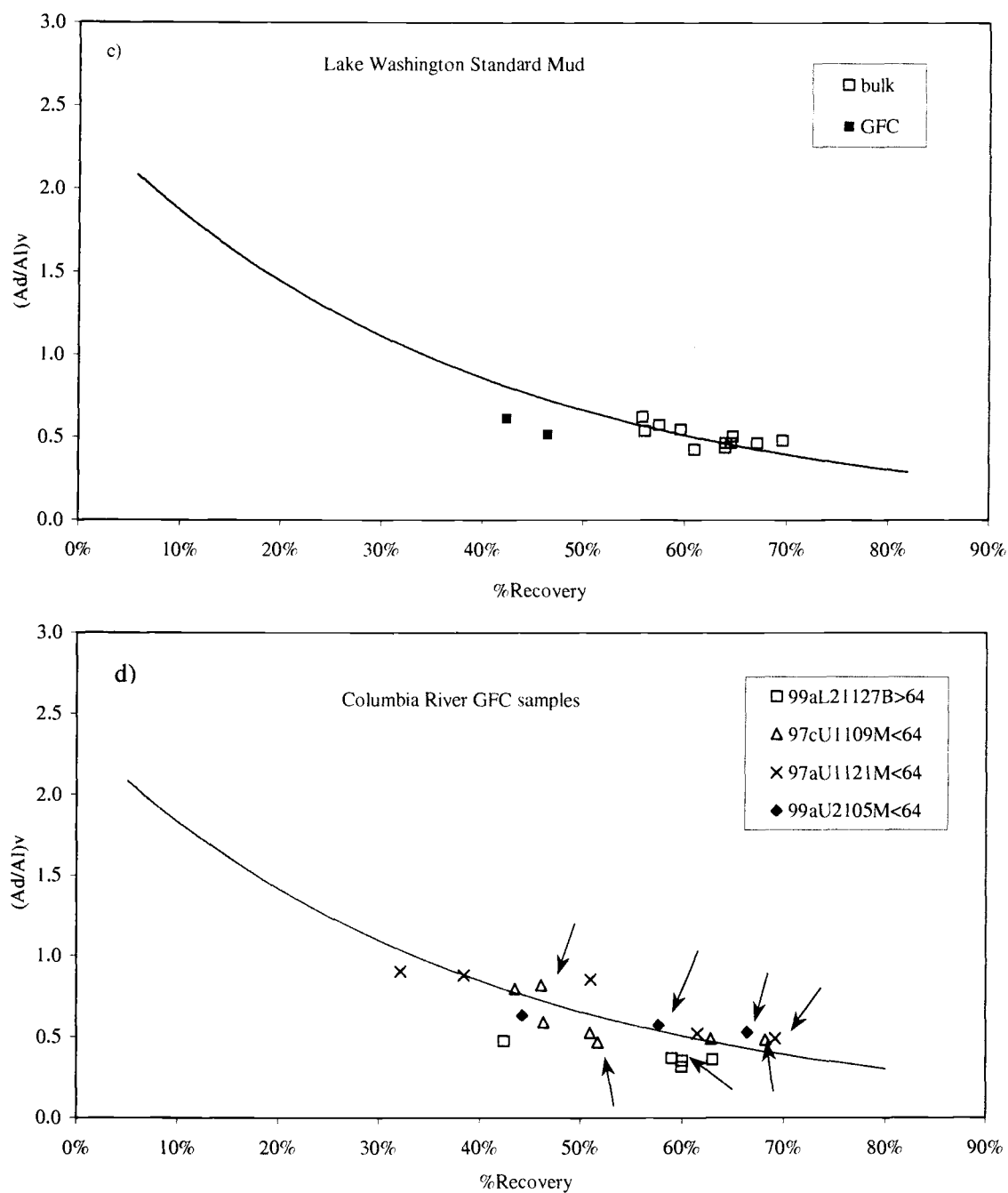
Figure A1 Continued
GFC: coarse glass fiber filter sample. The decreasing trend in plots c) and d) is that of plot a). Arrows in plot d) point to homogenized solid samples that were used as reference to assess for the impact of GFC on $(Ad/Al)_v$ data
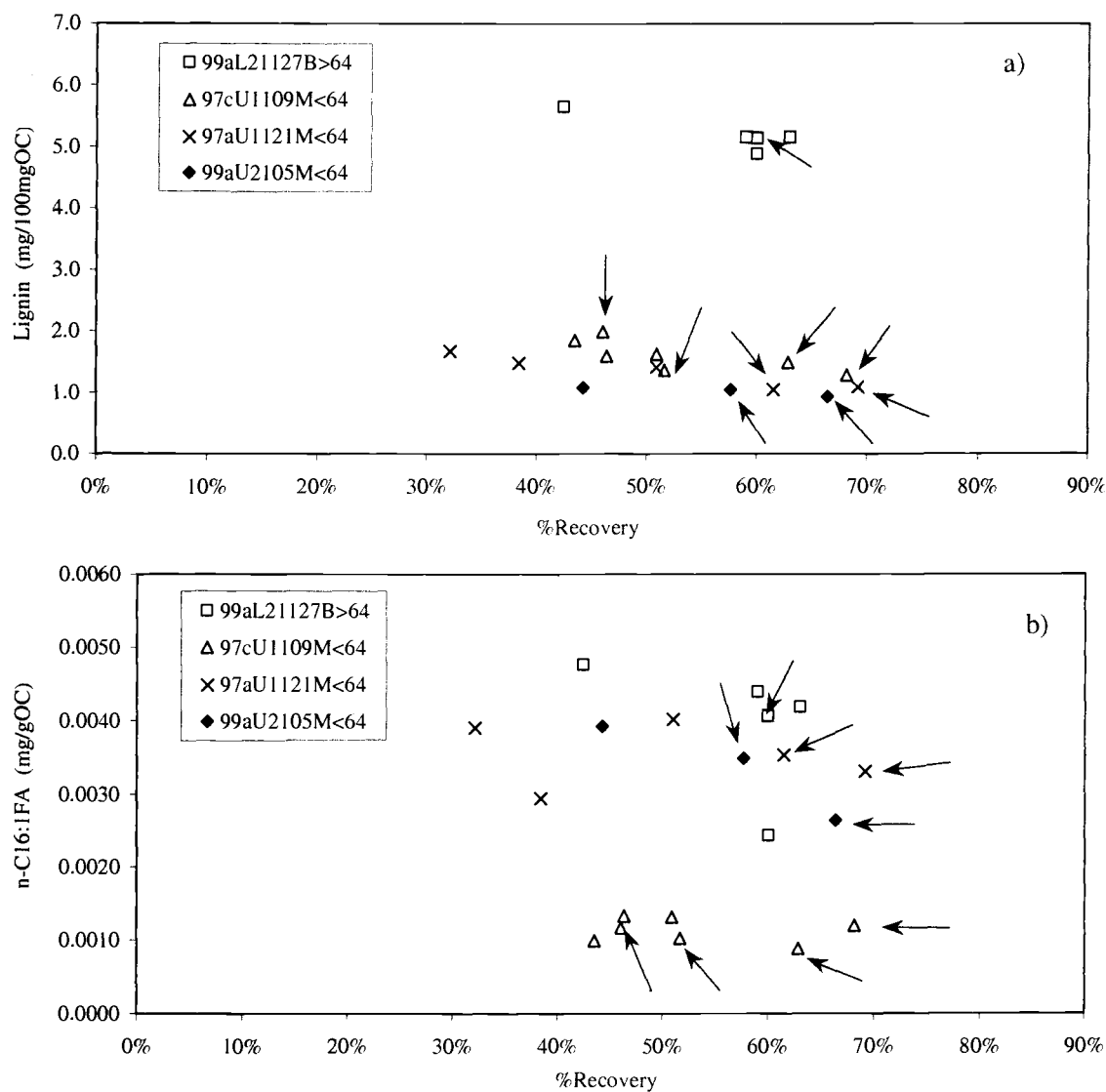
Figure A.2 Lignin and non-lignin parameters sensitivity to GFC filters
Tests on sensitivity of a) lignin content (allochthonous OM tracer) and b) n-FA
concentration (autochthonous OM tracer) showed no corruption of the data due to the
use of GFC filters. The other lignin parameters and other n-FA used in this thesis did
not show any change either. Arrows in plot point to homogenized solid samples that
were used as reference to assess for the impact of GFC

compounds including the recovery tracer: Et-Van. However, this experiment did not result in %Reco as low as those observed with actual samples, possibly implying that SPM quality and/or the quantity of $Si(OH)_4$ present in the medium may be important (small GFC filters were used). Second, higher $(Ad/Al)_v$ values were measured for GFC blanks as compared to CuO blanks. This clearly proved that the decomposition tracer was corrupted by the presence of the filter and therefore useless.
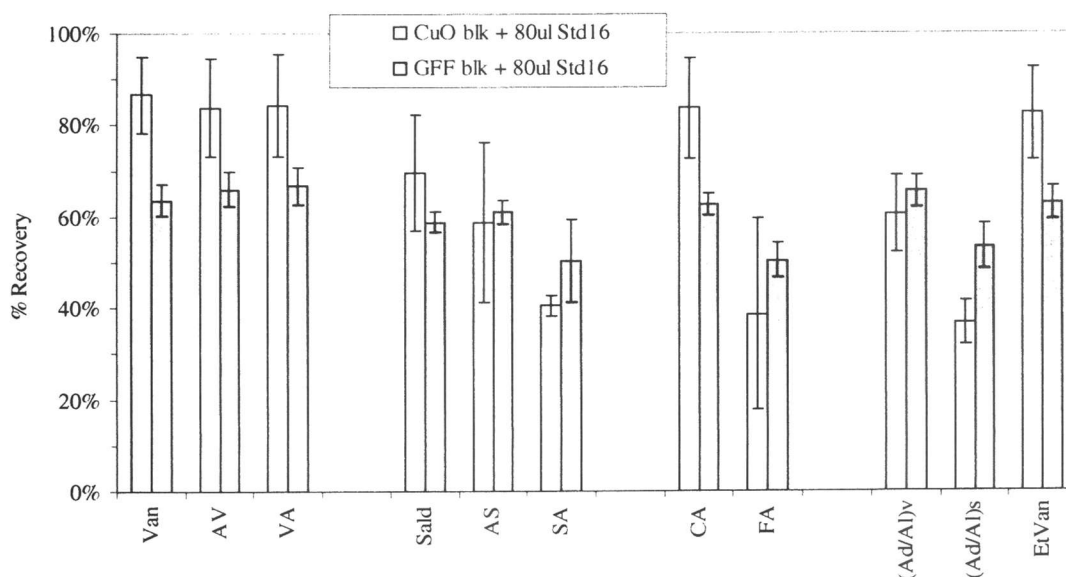

Figure A3 Impact of GFC filter on percentage recovery
A known amount of Std16 was introduced in the minibombs fallowing the CuO oxidation instead of solely Et-Van. Average results of duplicates are shown here (n=2). Compounds abbreviations are those of Figure 2.2. Error bars are the percentage variation between duplicates

Further evidence that POC quality makes a difference is provided by results from Fraser River samples on GFC. These samples [Figure A1 a)] did not display recoveries as low as those from Columbia River. They fit the decreasing trend but stayed within the range (50-70%) observed for homogenized solid samples from the Columbia River. Differences in SPM quality (i.e., composition) could be an explanation as more frequent and more abundant emulsions were noticed with Columbia River samples. It would have been interesting to analyze several homogenized solid samples from the Fraser River in order to put them into

perspective with GFC samples and with solid samples from the Columbia River. The only sample available displayed slightly higher %Reco than GFC samples (but not as marked as for the Columbia River), therefore enforcing the idea that the use of GFC filters for lignin analysis by CuO oxidation induces low recovery.

Analysis of my entire data set showed no evidence of difference in %Reco due to the nature of the compounds (i.e. acids versus aldehyde or ketone). However, the size of the phenolic rings side-chains seemed to have its importance: phenols with smaller side-chains like vanillyl phenols, showed higher %Reco than phenols with larger side-chains such as S or C phenols.

At that point, another hypothesis was tested that could explain the low %Reco obtained. As mentioned at the beginning of this chapter, V and C phenols were present in acidic and aldehydic forms. What if, under certain conditions, Et-Van was converted into its acidic form (Et-VA)? This would explain the low Et-Van recovery and would also lead to an overestimation of the lignin content (Equation 4). The presence of Et-VA could be evaluated using GC-FID results. The injection of an Et-VA standard would allow the determination of the compound retention time and RRF. Based on these data, the presence of this 'artifact' in a sample could be investigated and its concentration calculated. Instead, ET-VA presence was investigated by injecting GFC and solid samples of low to very low %Reco into the GCMS and by carefully studying their peaks. Based on fragments obtained with Van, VA and Et-Van, I investigated for probable specific peaks that should result from the presence of Et-VA. None of these peaks were observed, suggesting that correction for the low recovery problem in samples could be done confidently using Et-Van as a yield tracer. However, if aldehydes were significantly converted into acids during the CuO oxidation process, it was likely to happen during the reaction phase (i.e., bombing). In this case, introduction of Et-Van post-bombing would not trace the CuO oxidation products perfectly.

Table A1 CuO oxidation results for Columbia River samples on GFC filter
CuO oxidation results for samples artificially put onto GFC filters. B, M: bottom and
middle water sample. Details of date and location of collection can be found in
Appendix V. Lig, Λ: lignin content in mg/g and mg/100mgOC. S, V, C: in mg/g

	Lig	Λ	S/V	C/V	%Vo	%So	Vadal	Sadal	S	V	C
99aL21127 B>64											
no GFC	1.63	5.15	0.39	0.12	16.48	20.56	0.35	0.25	0.422	1.08	0.134
GFC	1.55	4.89	0.33	0.04	18.43	22.70	0.31	0.22	0.375	1.13	0.048
GFC	1.64	5.16	0.34	0.06	18.34	22.65	0.36	0.25	0.399	1.16	0.074
GFC	1.64	5.17	0.37	0.07	19.05	22.49	0.37	0.23	0.423	1.13	0.084
GFC	1.79	5.66	0.46	0.09	19.52	20.97	0.47	0.20	0.536	1.16	0.102
97aU1121 M<64											
no GFC	0.29	1.09	0.42	0.09	18.08	26.73	0.49	0.42	0.081	0.19	0.017
no GFC	0.28	1.05	0.41	0.09	17.93	23.16	0.52	0.52	0.077	0.19	0.018
GFC	0.38	1.41	0.51	0.12	20.31	29.78	0.85	0.56	0.118	0.23	0.028
GFC	0.45	1.67	0.48	0.10	20.64	29.74	0.90	0.63	0.136	0.29	0.029
GFC	0.40	1.47	0.43	0.09	20.95	27.33	0.88	0.88	0.112	0.26	0.023
97cU1109 M<64											
no GFC	0.30	1.99	0.32	0.07	25.52	26.42	0.82	0.31	0.069	0.21	0.015
no GFC	0.20	1.36	0.30	0.06	21.06	27.46	0.47	0.37	0.045	0.15	0.009
no GFC	0.22	1.49	0.29	0.06	20.19	27.42	0.49	0.47	0.048	0.17	0.010
no GFC	0.19	1.28	0.29	0.07	15.69	19.34	0.48	0.36	0.041	0.14	0.009
GFC	0.28	1.84	0.37	0.08	25.57	25.81	0.80	0.32	0.071	0.19	0.015
GFC	0.24	1.63	0.31	0.06	20.54	28.88	0.52	0.50	0.056	0.18	0.011
GFC	0.24	1.59	0.25	0.03	19.35	27.26	0.59	0.65	0.047	0.19	0.006
99aU2105 M<64											
no GFC	0.31	0.94	0.46	0.09	19.93	28.27	0.53	0.36	0.092	0.20	0.018
no GFC	0.35	1.05	0.50	0.10	18.13	29.70	0.57	0.36	0.109	0.22	0.023
GFC	0.36	1.08	0.41	0.08	20.79	29.34	0.63	0.67	0.099	0.24	0.020

Table A1 Continued

n-fatty acids: tetradecanoic acid (n-C14FA), hexadecenoic acid (n-C16:1FA) and hexadecanoic acid (n-C16FA). Diacids: butane-1,4-dioic acid (C4DA), 2-butene-1,4-dioic acid (C4:1DA). Phenol: p-hydroxyphenylglyoxylic acid (Pg). Concentrations are in mg/gOC

	C4DA	C4:1DA	Pg	n-C14FA	n-C16:1FA	n-C16FA	3,5diBA /V	%Rec
99aL21127 B>64								
no GFC	0.0026	0.0028	0.0023	0.0036	0.0024	0.0010	0.051	60.0
GFC	0.0028	0.0037	0.0019	0.0051	0.0041	0.0039	0.033	60.0
GFC	0.0035	0.0037	0.0018	0.0050	0.0042	0.0038	0.032	63.0
GFC	0.0038	0.0027	0.0016	0.0060	0.0044	0.0057	0.032	59.0
GFC	0.0103	0.0019	0.0014	0.0075	0.0048	0.0104	0.036	42.4
97aU1121 M<64								
no GFC	0.0014	0.0020	0.0011	0.0043	0.0040	0.0018	0.081	69.2
no GFC	0.0008	0.0023	0.0015	0.0040	0.0039	0.0010	0.090	61.5
GFC	0.0029	0.0034	0.0023	0.0033	0.0033	0.0008	0.137	51.0
GFC	0.0036	0.0047	0.0028	0.0036	0.0035	0.0009	0.144	32.1
GFC	0.0020	0.0053	0.0021	0.0039	0.0029	0.0008	0.149	38.4
97cU1109 M<64								
no GFC	0.002	0.0010	0.0008	0.0011	0.0012	0.0004	0.081	46.1
no GFC	0.0007	0.0007	0.0003	0.0013	0.0010	0.0011	0.061	51.7
no GFC	0.0006	0.0009	0.0003	0.0012	0.0010	0.0007	0.062	62.8
no GFC	0.0008	0.000	0.0001	0.0011	0.0009	0.0015	0.041	68.2
GFC	0.0016	0.0009	0.0006	0.0012	0.0013	0.0006	0.088	43.5
GFC	0.0005	0.0012	0.0005	0.0011	0.0012	0.0007	0.074	50.9
GFC	0.0011	0.0009	0.0002	0.0014	0.0013	0.0017	0.048	46.4
99aU2105 M<64								
no GFC	0.0033	0.0034	0.0039	0.0039	0.0026	0.0008	0.096	66.4
no GFC	0.0032	0.0038	0.0041	0.0048	0.0035	0.0010	0.099	57.7
GFC	0.0020	0.0073	0.0033	0.0071	0.0039	0.0011	0.085	44.3

Table A2 Lignin results for LWSM on GFC filter
Lig, Λ: lignin content in mg/g and mg/100mgOC. Other concentrations are in mg/g

	Van	AV	VA	SAld	AS	SA	CA	FA	3,5diBA/V	%Rec
Sample 1	0.340	0.128	0.175	0.168	0.069	0.057	0.066	0.045	0.08	46.5
Sample 2	0.351	0.139	0.214	0.168	0.084	0.067	0.075	0.051	0.08	42.4
average	0.345	0.133	0.195	0.168	0.077	0.062	0.070	0.048	0.08	
c. var	2.2%	5.6%	14.2%	0.1%	14.2%	11.5%	8.4%	8.0%	2.9%	

	V	S	C	Lig	Λ	S/V	C/V	%Vo	%So	Vadal	Sadal
Sample 1	0.643	0.294	0.111	1.049	2.22	0.46	0.17	19.91	23.44	0.516	0.337
Sample 2	0.704	0.319	0.125	1.148	2.43	0.45	0.18	19.70	26.39	0.612	0.397
average	0.674	0.306	0.118	1.098	2.322	0.455	0.176	19.81	24.91	0.564	0.367
c. var	6.3%	5.8%	8.2%	6.4%	6.4%	0.5%	1.9%	0.7%	8.4%	12.0%	11.6%

Conclusion

Results clearly indicate that the presence of glass fiber filters resulted in a decrease of the %Reco and compromised the use of $(Ad/Al)_v$ as allochthonous OM decomposition tracer by artificially increasing it.. However, no effects on other quantitative lignin and non-lignin results were noticed, therefore allowing their use. It is not clear what precisely is happening chemically during the CuO oxidation process of samples collected on GFC filters. It seemed likely however, that a matrix effect due to the filter composition $[Si(OH)_4]$ was responsible for the problem encountered, potentially worsened by the quality of the POC analyzed. This constitutes a critical issue as GFC filters are widely used to collect SPM samples. Especially, the loss of the information about the sample state of decomposition is critical, particularly when the fate of the land-derived OM throughout the system is the main focus of the study.

NEW APPROACH TO ASSESS QUALITY/RELIABILITY OF LIGNIN DATA?

Throughout this thesis work, one question was constantly recurring: How can one be assured of the quality of the lignin results? Investigation of reproducibility constituted one test. But are there other simple ways to evaluate the validity of the data?

While analyzing lignin results, my attention was brought upon a conspicuous relationship between $(Ad/Al)_s$ and $(Ad/Al)_v$ ratios. As Figures A4 and A5 show, $(Ad/Al)_v$ and $(Ad/Al)_s$ increased in a well-correlated fashion, with coefficient of correlation (r^2) ranging from 0.60 to 0.80 for my data and 0.44 to 0.90 for results from other studies (see references therein). How does this prove anything about the quality of the data? As a matter of fact, none of the data from samples corrupted during the laboratory work [some examples: circled data in Figure A4 e)] fit this trend. Another interesting aspect was that the slopes obtained for bulk samples on GFC filters were similar, independently of their origin [Figures A4 a), b),c) and d)]. However, size-fractionated samples gave a slightly lower slope and correlation, respectively 0.53 and 0.57. It appeared that the conspicuous relationship between $(Ad/Al)_v$ and $(Ad/Al)_s$ ratios might reveal information about data quality. This feature did not appear related to sample origin as it was observed for samples from the Columbia, Fraser, Amazon and other US continental rivers [Figures A4 a), b), d) and A5 b)] as well as for Lake Washington [Figure A4 c)] and for wood samples [Figure A5 a)]. The property seemed also to be independent of sample type as it was observed for both GFC samples and homogenized solid samples. Further consideration of the size-fractionated samples revealed that fine and coarse SPM did not plot in the same areas and behaved similarly for both Columbia River and Amazon River samples [Figure A4 e) and Figure A5 b)]. Coarse material displayed lower $(Ad/Al)_{s\ or\ v}$ ratios, which fit the idea of larger vascular plant debris being less degraded than smaller ones. At last, data presented in Figures A4 and A5 were

obtained by different researchers using slightly different methods, which therefore discarded a method-related feature.

One disturbing fact however, is that the slopes of the relationships are not all the same and may vary depending upon the samples considered. Although the majority of the plots presented here have similar slopes, two of them [Figures A5 a) and b)] were different. It is not clear what drove these slopes. If all these relationships were natural occurrence it would therefore be hard to objectively pick out an artifact. More work has to be done in order to discover if that relationship could be used to discriminate good from bad results or if it has even more potential. What was certain was that this feature was not fortuity and, at least within my dataset, corresponded with good results.
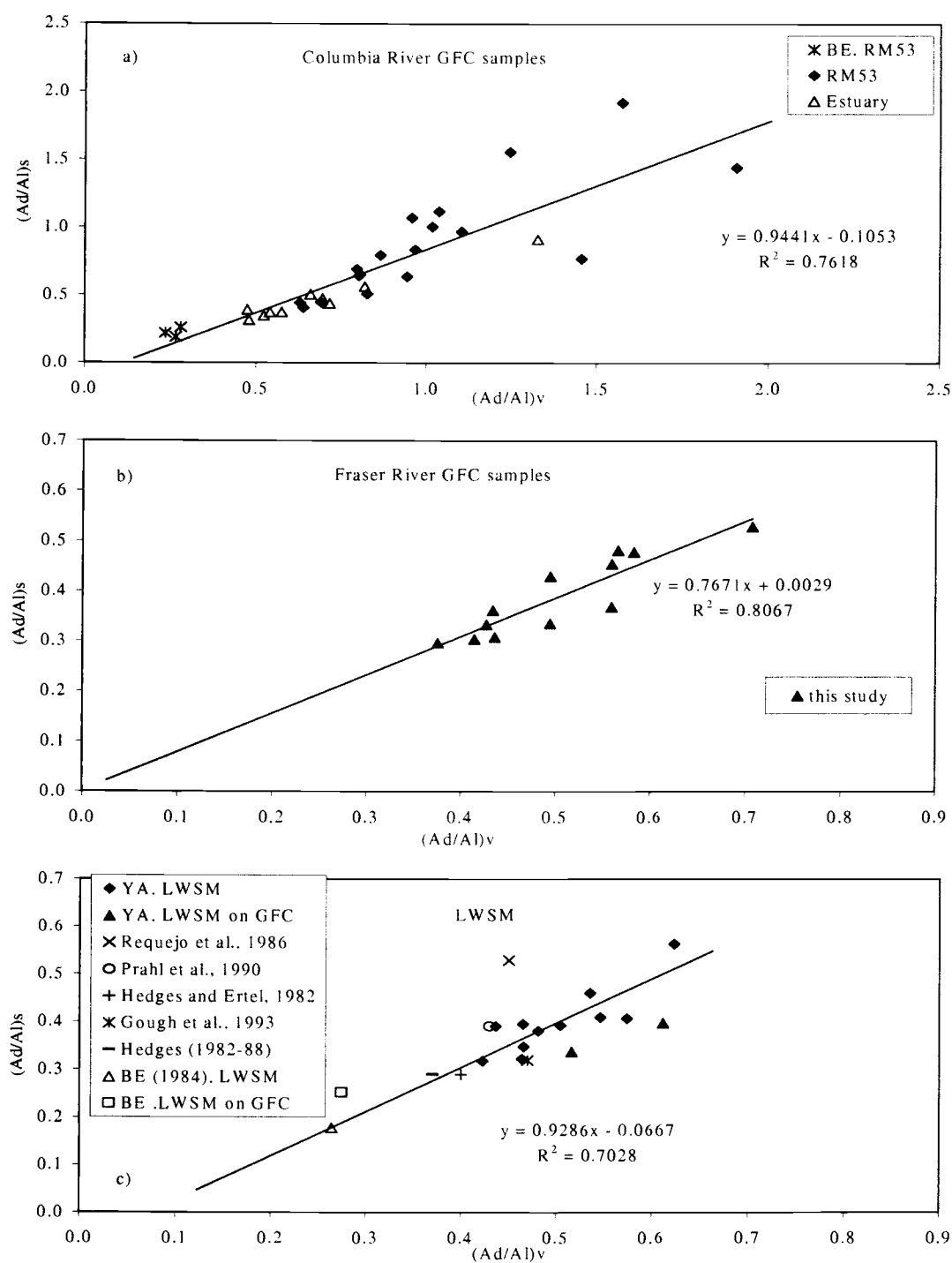
Figure A4 Correlation between $(Ad/Al)_v$ and $(Ad/Al)_s$ ratios
BE: Eversmeyer (1984) CR-RM53 unpublished data. Trend line in plot c) is for filled diamonds. YA: this study. LWSM: Lake Washington Standard Mud
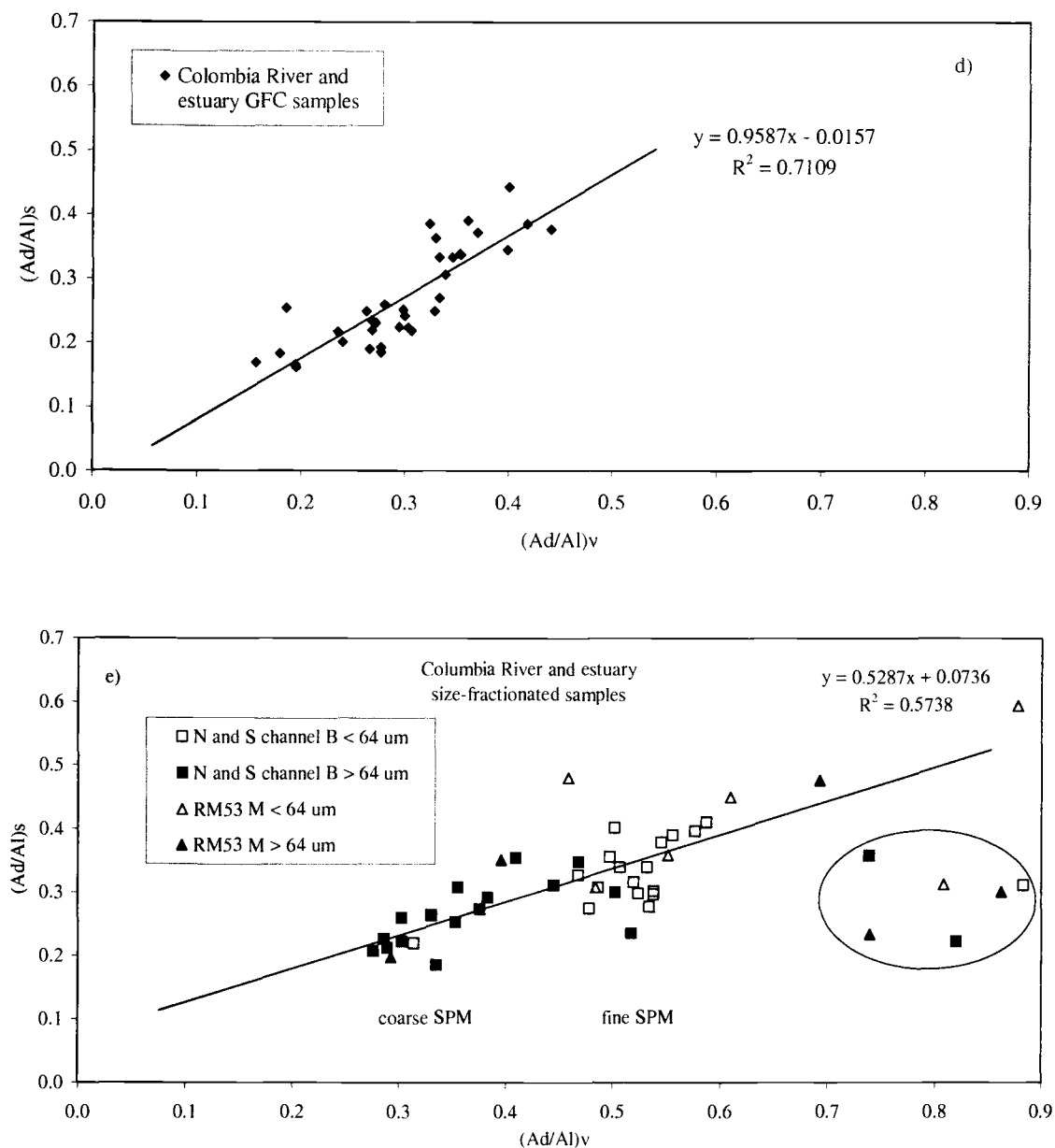
Figure A4 Continued

Plot d) are archived, unpublished data from Eversmeyer. Samples are from the Columbia River (except RM53) and estuary. e) Samples are from the North and South channels of the Columbia River estuary. Coarse and fine samples are define as > 64 μm and 64 μm < size < .45 μm respectively. Circled data are examples of bad data, which did not fit the trend
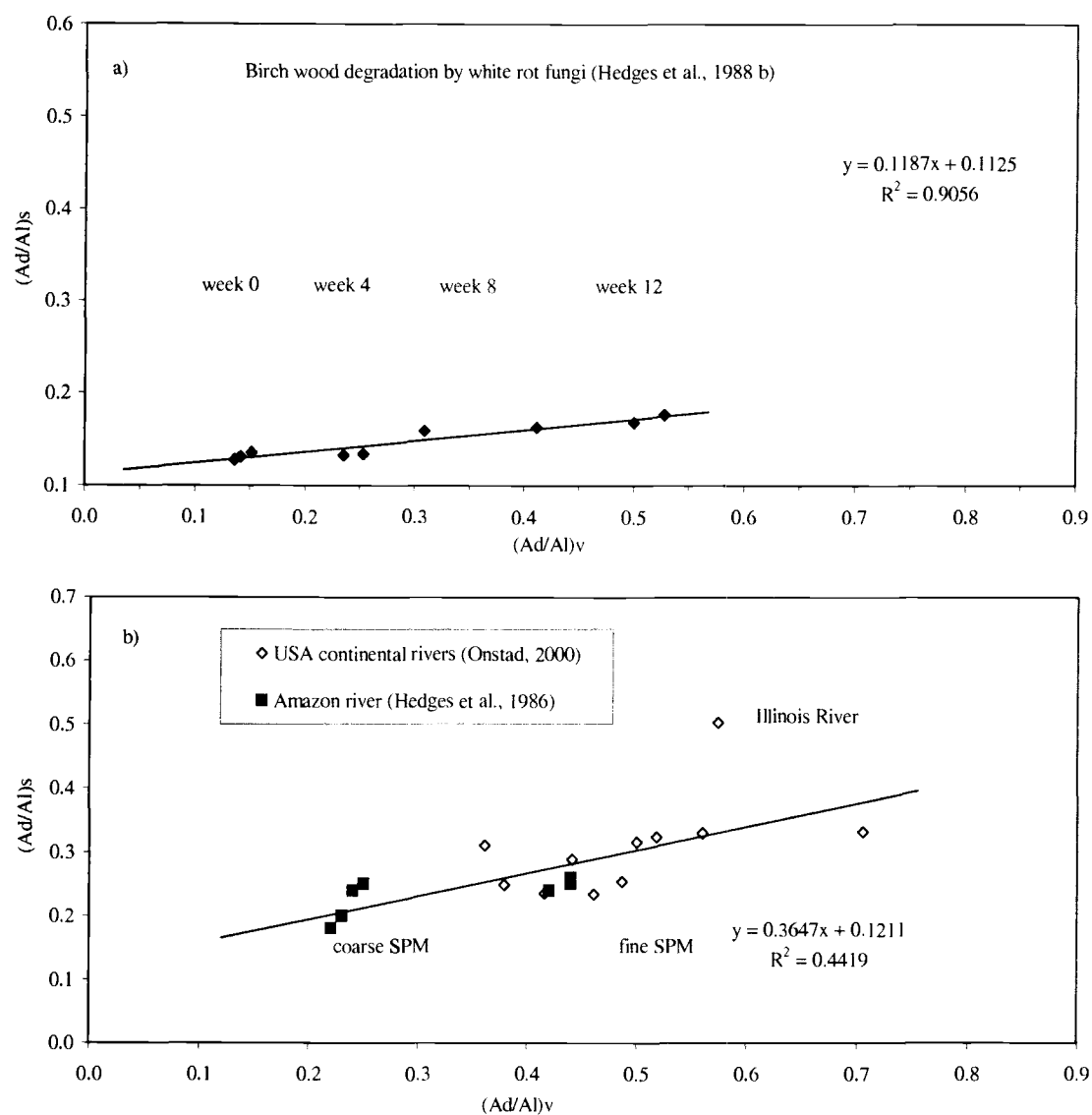
Figure A5 Correlation between $(Ad/Al)_v$ and $(Ad/Al)_s$ ratios
The correlation can be observed with results from other studies: a) Wood samples
degraded by white rot fungi, Hedges et al. (1988 b); b) SPM samples from the
Amazon River, Hedges et al. (1986) and twelve US continental rivers, Onstad (2000)

RECOMMENDATION / PROBLEM SOLVING

About (Ad/Al)$_v$

The use of GFC filter to collect suspended particulate matter for lignin analysis induced low recovery (%Reco) and artificially increased (Ad/Al)$_v$ usually used as degradation index. The other parameters gathered from the GC analysis of CuO oxidized samples seemed not to be impacted by recovery issues. However, samples of low to very low %Reco should be discarded as the correction factor (Equation 4) in the compound concentration calculation increases, adding uncertainty. As a consequence, I advise: (1) to not use existing (Ad/Al)$_v$ data from CuO oxidation of GFC samples until further work as been done to throw some light on that issue, (2) avoid the use of GFC filters in future sampling if lignin work is to be done on those samples.

More testing would be necessary to elucidate precisely what was happening with the GFC filters. The interaction between some types of compounds (possibly phytoplankton-related) with the silica of the filter is a possibility but the source of the problem could be multiple. GFC filters are widely used to collect samples and therefore a method has to be found in order to use these filters even for lignin analysis. The solution could consist in a pretreatment of the GFC samples to dissociate the SPM load from the filter. This can possibly be done either by washing the sample out of the filter or by dissolving the filter. Gélinas et al. (2001) presented and discussed a method for the demineralization of particulate matter and recently deposited sediments using HCl and HF, and the recovery of labile OM dissolved during the process. This particular procedure would however not be suitable for large sets of samples as it would be very tedious and time consuming. Nonetheless, if such a method were to work, it would allow the use of already exiting samples on GFC filters. This method is sited as an example and might not be the best way to proceed

but could constitute a starting point for solving the GFC-lignin analysis issue. Other pretreatment techniques have yet to be elaborated. Other solutions should be investigated that could involve 1) a method of correction for the corrupted data or 2) the use of different types of filters. On a practical standpoint, the second option seems the fastest and easiest to search. Especially, the use of a filter which would allow direct measurements of lignin parameters without any other "manipulation" is suitable and would simplify a technique that is already complicated enough.

A new lignin degradation index

As previously stated, $(Ad/Al)_v$ results from samples collected on glass fiber filters should be discarded. This omission would deprive us from a very interesting and crucial information about the nature of the lignin content of the OM, i.e. its degree of degradation. To the best of my knowledge, no other index besides $(Ad/Al)_v$ has yet been formulated to evaluate the degree of lignin degradation. Keil et al. (1998) observed good correlation between $(Ad/Al)_v$ and both non-protein amino acid mole percentages and sum wt% of the deoxyaldoses (sugars) fucose and rhamnose, which they hypothesize could be used as potential diagenetic indicators. However, the use of these sugars is difficult given that they do not originate from a unique source. Indeed, their concentrations were found to increase in both degraded marine plankton (Cowie and Hedges, 1994; Hamilton and Hedges, 1988) and in degraded vascular plant material (Cowie and Hedges, 1984 a; Hedges and Weliky, 1989).

A close look at my results indicates that the ratio of 3,5-dihydroxybenzoic acid to total vanillyl phenols (3,5dBA/V) holds promise as a diagenetic index for lignin. When plotting 3,5dBA/V versus $(Ad/Al)_v$ for samples from the Fraser River and Lake Washington [Figures A6 a) and b)] a conspicuous trend was observed: 3,5dBA/V increased in direct proportion with $(Ad/Al)_v$. Archived, unpublished data from Eversmeyer B. for Columbia River and estuary samples [Figure A6 c)] showed
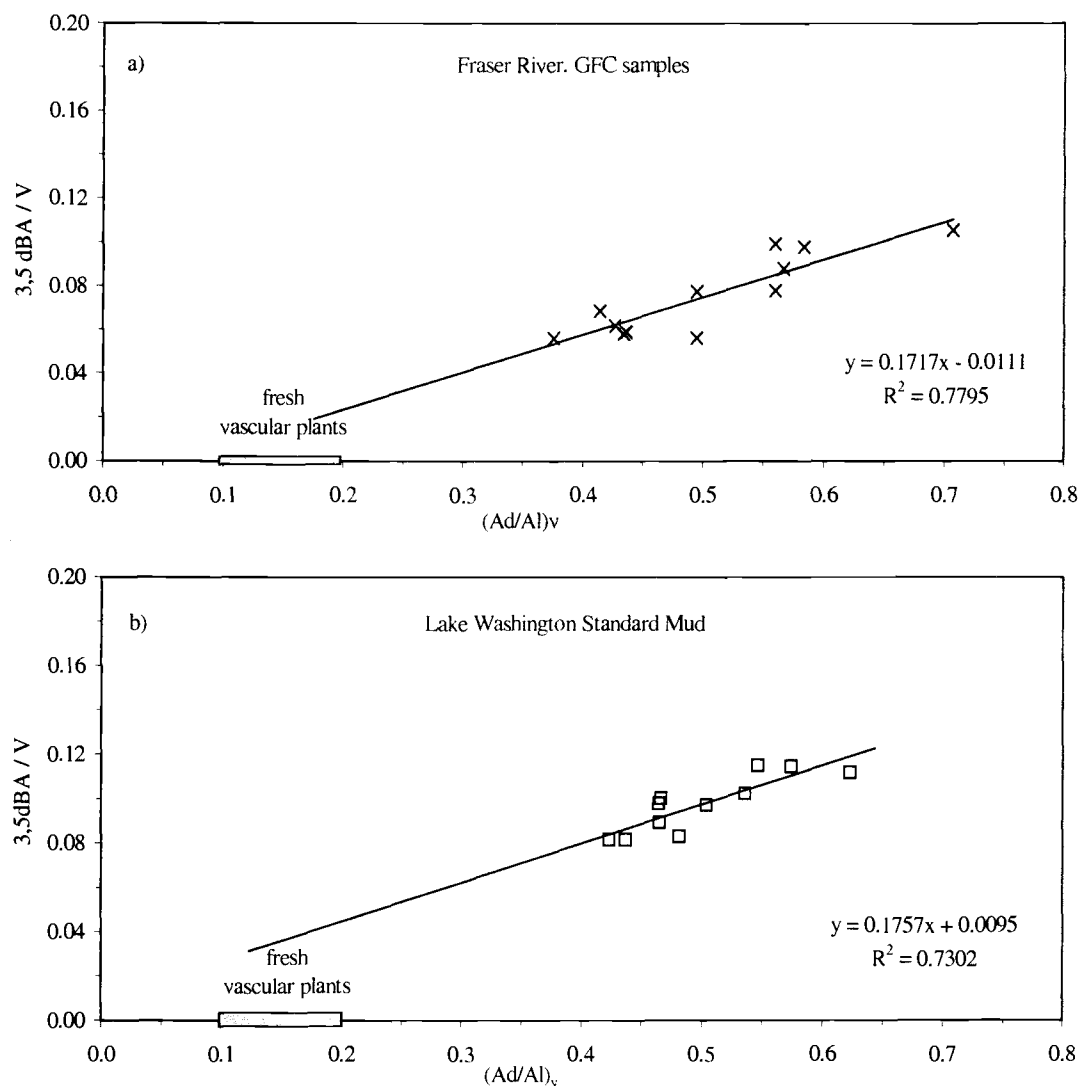
Figure A6 New index for lignin degradation level: 3,5dBA/V
Data in plots a) are GFC samples from the Fraser River and b) solid sample from
Lake Washington Standard Mud. "Fresh vascular plants" area based on Hedges et al.
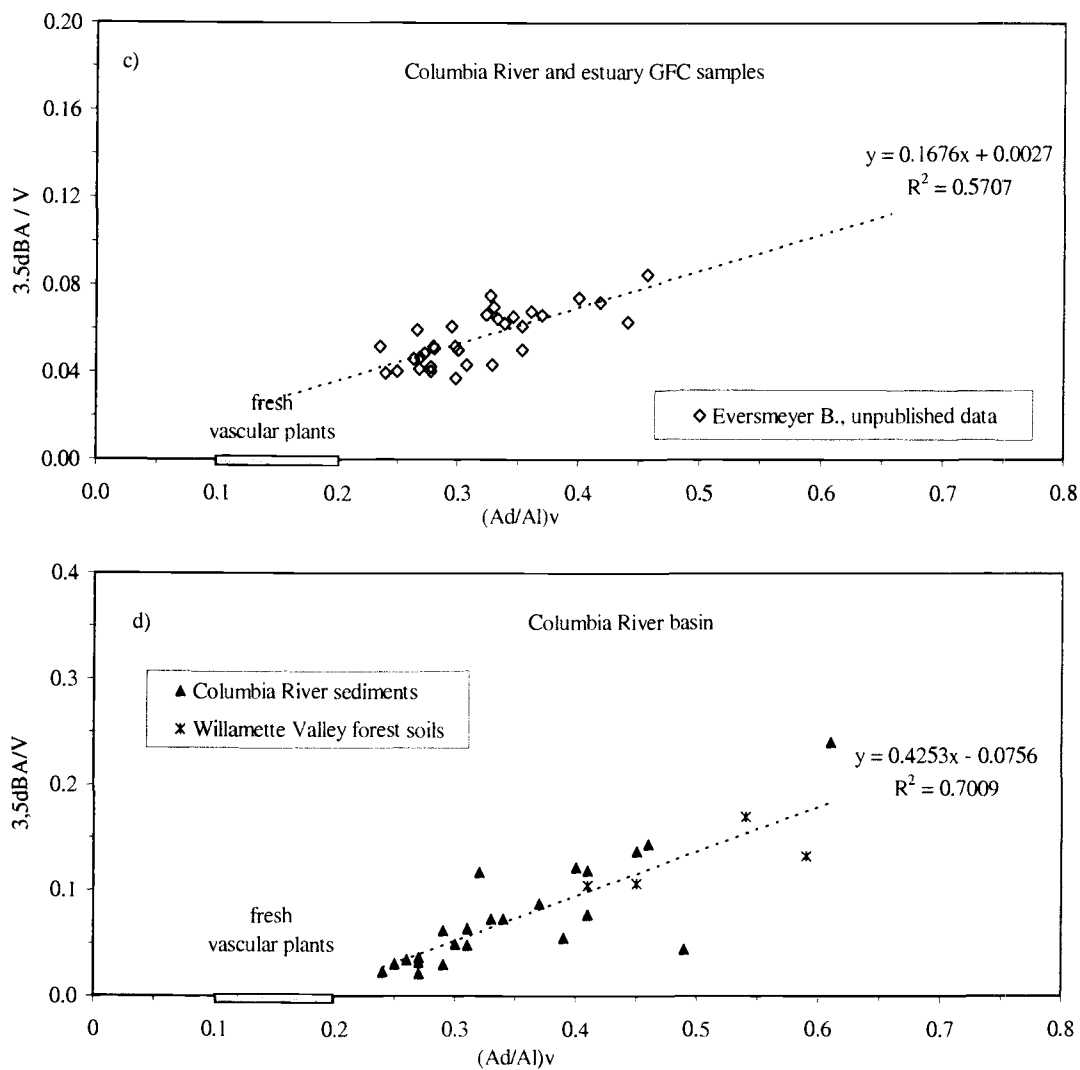(1982): 0.15 ± 0.05

Figure A6 Continued
c) Unpublished data from B. Eversmeyer obtained in our lab; d) Columbia River basin sediment samples and Willamette Valley forest soils samples. Data from Prahl et al. (1994)

the same trend. Interestingly, these three plots obtained for samples of different origin, composition, analyzed by different researchers with slightly different methods and in different years, revealed relatively similar slopes and intercepts, therefore arguing that this feature was not an artifact of my laboratory work but a real occurrence. Results from Prahl et al. (1994) about sediment samples collected throughout the Columbia River basin showed this feature as well [Figure A6 d)] but with a different slope. Results from local forest soils (Willamette Valley deciduous and coniferous forest) fit this trend as well (Prahl et al., 1994).

There was however, a potential draw down to the hypothesis that 3,5dBA/V could be used as a tracer of lignin degradation level. To be used as a tracer the compounds implicated in this ratio should be exclusively encountered in terrestrial vascular plants. Although vanillyl phenols are found solely in vascular plants, this is not necessarily the case for 3,5dBA. Indeed, earlier work has shown some brown, green and red macroalgea as well as some phytoplankton to produce significant yields of 3,5dBA upon CuO oxidation (Goni, 1992. p. 312). Those were marine species, therefore out of concern for upriver samples (RM53) but they eventually could be present in estuary samples. Studying their area of distribution would be useful at that point. Based on data for these marine organisms, one could also eventually speculate that fresh-water equivalent could exist. However, if such freshwater phytoplankton species producing 3,5dBA were to exist in the Columbia River, one might expect Chla and 3,5dBA concentration results to be correlated. No correlation was found between 3,5dBA and Chla concentration for the Fraser River, the Columbia River and estuary GFC samples. The intercepts values of Figures A6 are likely to be due to the fact that fresh vascular plant tissues contain 3,5dBA and V in a proportion that spans a range of values (0.001-0.004?) in the same way $(Ad/Al)_v$ does (0.15 ± 0.05, Hedges et al., 1982).

The problems encountered while using SPM samples collected on GFC filters and discussed in this part is particularly relevant as this type of filter is widely used to

rapidly collect SPM samples. That technique allows sampling at short time intervals as compare to ultrafiltration, which requires 2 hours per sample (5 min for collection, 15 min for separation of > 64 μm fraction, 50 min for ultrafiltration and 50 min to centrifuge), being therefore impractical for high resolution, tidal time series sampling. My thesis provides, to the best of my knowledge, the first report about the problem of using GFC filters for lignin analysis by CuO oxidation. However, personal communication with Drs. John Hedges and Miguel Goni revealed that low recovery when using GFC sample was not restricted to my work but was experienced by other researchers but never formally reported previously.

From my results, it appeared possible that (3,5dBA/V) may constitute a way to gain information about the degradation state of lignin, a detail that was lost from samples analyzed on GFC owing to corruption of the $(Ad/Al)_v$ data. However, further investigation will be required to establish a method by which 3,5dBA/V could be used effectively as an index of lignin degradation. Understanding and solving this problem is of primary importance: my work represents a first step in that direction.

SUMMARY

1. While previous studies demonstrated that the degradation indicator $(Ad/Al)_v$ was sensitive to O_2 intrusion and oxidation temperature during the CuO process, my results showed that high $(Ad/Al)_v$ and low %Reco values are obtained from the CuO oxidation of samples collected on glass fiber filters. A careful study of the results from samples of divers origins as well as tests conducted on blanks and solid samples seemed to attribute this problem to the composition of the filters $[Si(OH)_4]$ and the quantity of this material in the reaction medium. Furthermore, the quality of the sample's SPM seemed in some cases to worsen the problem.

2. The direct correlation observed between $(Ad/Al)_s$ and $(Ad/Al)_v$ ratios could possibly allow the differentiation between good and bad data.

3. The ratio of 3,5-dihydroxybenzoic acid to vanillyl phenols (3,5dBA/V) shows promise for assessing the state of lignin degradation for both homogenized solid and GFC samples. Its use would be particularly useful in cases where $(Ad/Al)_v$ has been compromised owing to analysis of samples on GFC filters.

4. Et-Van might not be the best way to trace effectively the CuO oxidation products. Tests on blank samples in which a standard made of sixteen compounds was introduced after the oxidation and prior to the extraction phase, showed recovery differences between the different compounds and Et-Van. As a consequence, some compounds will be overestimated and other underestimated. Furthermore, if the conversion of aldehydes to acids takes place during the oxidation phase Et-van would not be present in the sample to assess for that change.

APPENDIX B

DAILY VARIABILITY OF LIGNIN CONTENT IN SPM:
A TIDE-DRIVEN FEATURE

Earlier studies of the Columbia River showed tidally driven changes in the river features. Covert (2001) described high SPM content during ebbing (high flow) and low SPM during flooding (low flow), as well as opposite patterns for Mn/Al and Chl-a/POC (12 hrs time series). Although not explicitly linked to a tidal effect, Sullivan et al. (2001) noticed the same relation between SPM and Chl-a concentration and river flow. Based on these results, a legitimate question would be to ask if land-derived POM carried by the river would respond as well to tide-driven flow changes and if these changes are sensitive to environmental conditions (i.e., season). To address this question, two 12 hours time series were collected in October 1997 and February 1998 and analyzed by CuO oxidation.

The observation of Figures B1 a) and b) for the winter sampling clearly shows that lignin concentration increased by a factor of two from a fairly uniform concentration during the onset of the first ebb event (12 pm) to the following slack period. The maximum of lignin content was achieved shortly after the maximum in flow. Interestingly, lignin concentration per gram of sediment (Lig) and per gram of OC (LIG) both increased but in slightly different ways. The highest and fastest increase concerned LIG while the Chla concentration remained stable, barely reaching the range (10-30 mg/gOC) typical of fresh phytoplankton (Figure B1 c). This implies that the majority of the POM resuspended from the bottom was land-derived. Values obtained for $(C/N)_{at}$ and %Al confirmed this feature. This is not surprising as one might expect labile phytoplankton OM to be rapidly recycled and therefore less preserved in sediment as compared to more refractory land-derived material. Furthermore, winter is a low light low nutrient period therefore unsuitable for phytoplankton growth. The small decrease in $(C/N)_{at}$ observed could be explained by a different type of soil-derived matter that composed the resuspended material as
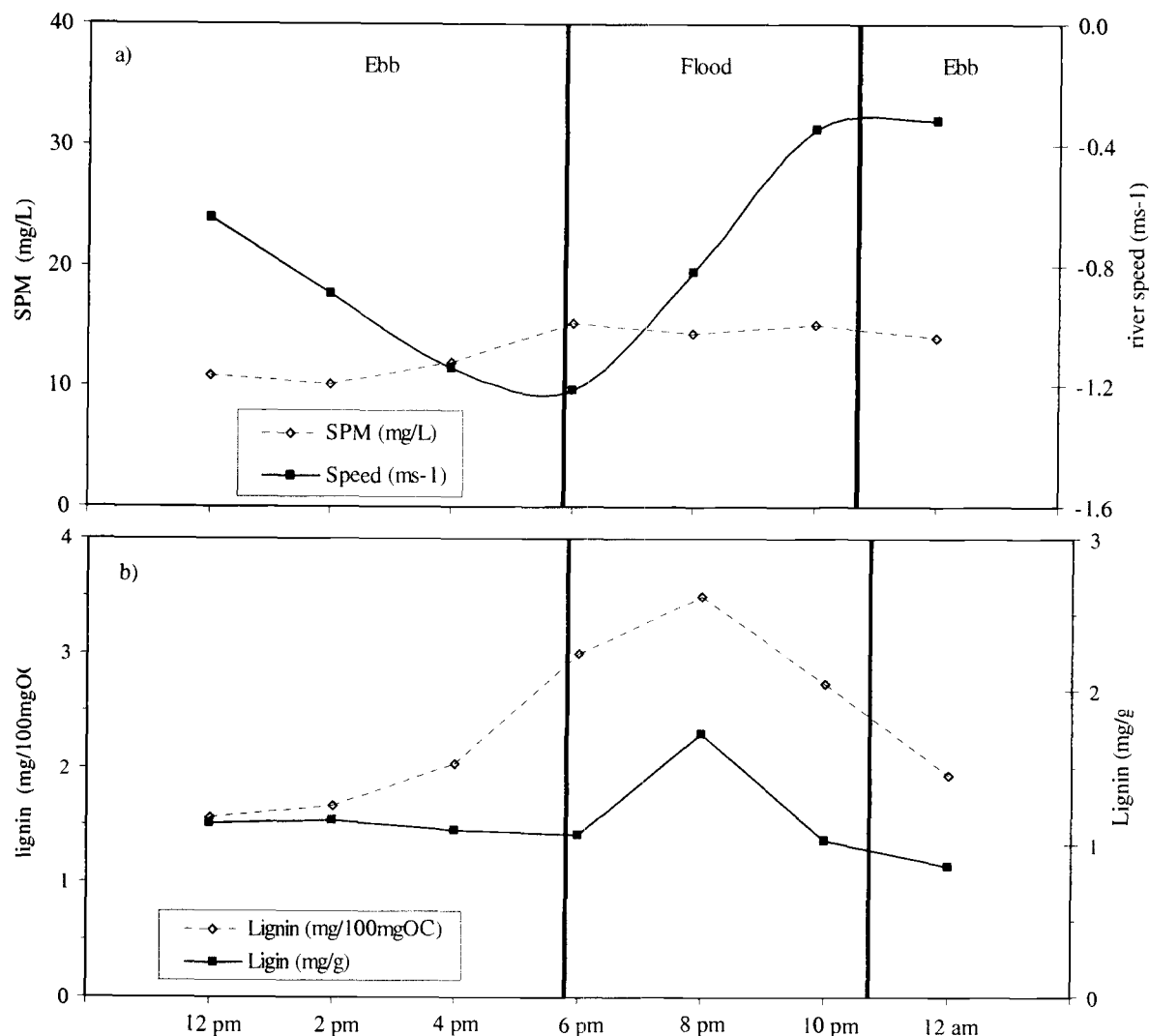
Figure B1 Winter 1998, 12 hours time series
a) SPM content and river speed; and b) lignin concentration at RM53. Samples were collected on GFC filters

compare to what was already in suspension. However, $(C/N)_{at}$ data stayed well within the range usually observed for soils (10: Bowen, 1979; 8-12: Stevenson et al., 1972; 7-15: Hedges and Oades, 1997b; Willamette basin forest soils: 11.1-13.4 deciduous; 13.7 coniferous, Prahl et al., 1994) and under that of fresh vascular plant tissues (20-400, Hedges et al., 1986; Hedges et al., 1997). Not surprisingly, most of the resuspended matter is mineral like. %POC decreases (diluted by organic-poor

matter) towards values found in soils (1.6-3.3: Prahl et al., 1994) while %Al slightly increases [Figure B1 d)]. As previously observed in this study, the lignin and SPM concentration are flow-dependent. Higher flow results in higher lignin content owing to resuspension of sedimentary material. This is not necessarily the case yearlong and environmental/seasonal conditions can results in different features.
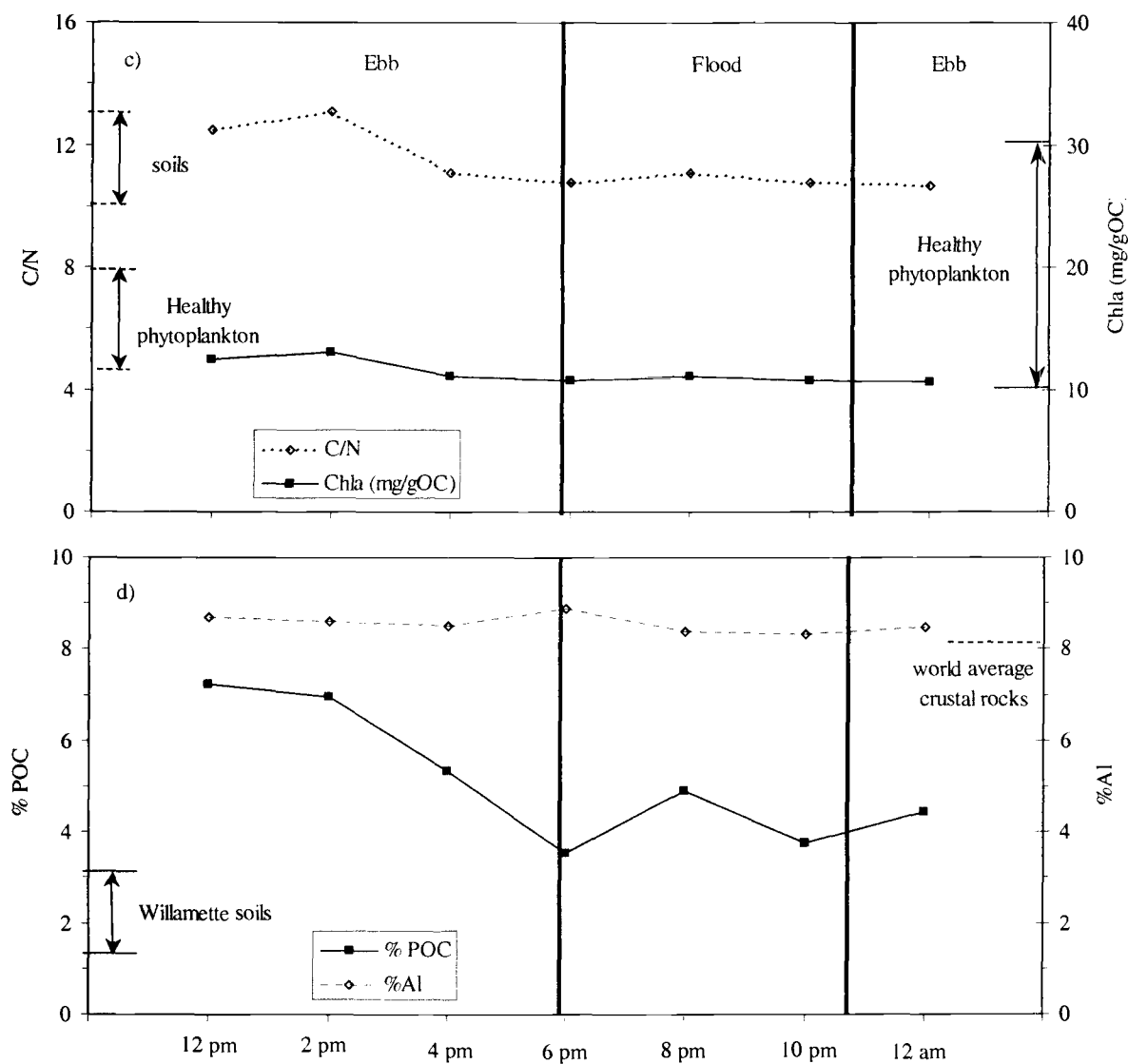

Figure B1 Continued
c) (C/N) at and Chl-a concentration; and d) %POC and %Al by weight at RM53. Samples were collected on GFC filters

During the fall 1997 time series, the SPM was quite high and showed a strong correlation with the river's flow, decreasing from 36 mg/L during the ebb down to 17 mg/L at the slack period [Figure B2 a)]. The lignin content of the samples only barely increased from 2.6 to 3 mg/100mg OC, reaching its maximum when the SPM and the flow were at their minimum. Meanwhile the lignin content per gram of sample remained constant [Figure B2 b)]. The likely explanation for this feature
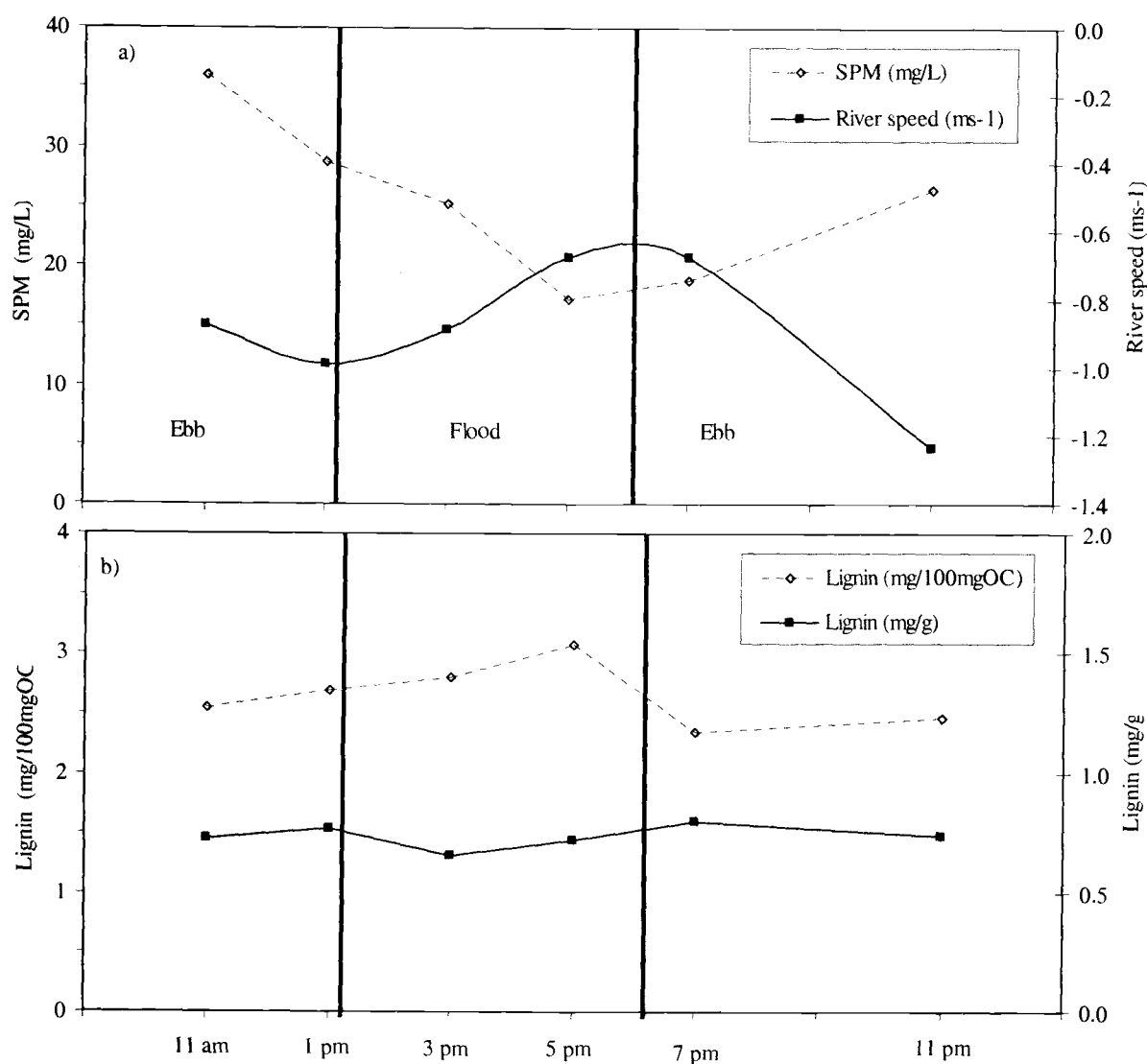

Figure B2 Fall 1997, 12 hours time series
a) SPM content and river speed; and b) lignin concentration at RM53. Samples were collected on GFC filters

is that contrary to winter months, land-derived matter does not constitute the main source of OM during early fall. Instead, autochthonous OM dominates. However the story here is a little more complex than during winter. The increase of the phytoplankton contribution at low flow is documented by both Chla measurements that increase from 7 to 16 mg/g OC (within the range expected from healthy fresh phytoplankton) and by $(C/N)_{at}$ data, which decreased from soil-like values (11) down
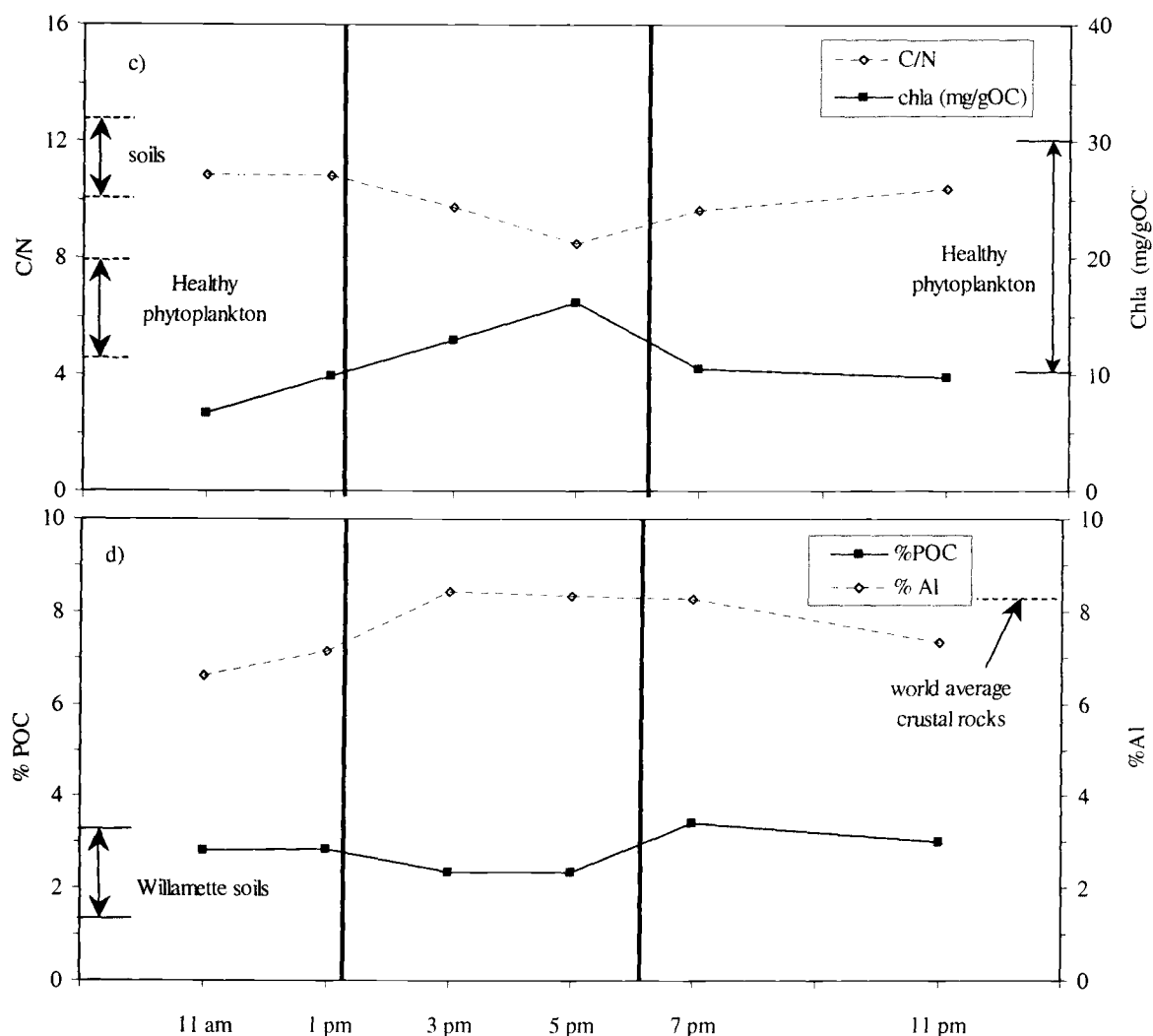

Figure B2 Continued
c) (C/N) at and Chl-a concentration; and d) %POC and %Al by weight at RM53.
Samples were collected on GFC filters

to values (8.5) close to those typical of fresh phytoplankton [6.6: Redfield et al., 1963; 5-8: Hedges and Oades, 1997 b; Hedges et al., 1997 a), Figure B2 c)]. Fall is a period that follows the spring/summer bloom when a huge amount of phytoplankton is typically present in the Columbia River. Therefore, it is likely that a consequent standing stock of phytoplankton is present. The variation observed in Chla content (i.e., phytoplankton) potentially results from the strong vertical mixing at high flow during which the cells are distributed throughout the water column. Under lower flow, the phytoplankton can stay in the photic zone and multiply while heavier particles that were resuspended at high flow settled back onto the sediment. Material injected to the water column by an increase of the river flow is mainly of soil origin as suggested by both $(C/N)_{at}$ and %Al data. However, while heavier land-derived organic-rich particles settled fast (suggested by the lignin plot), mineral-rich fine particles stayed longer in suspension. This is suggested by the %Al data that took time to increase during high flow, remained high even at low flow then slowly started to decrease [Figure B2 c)].

The observation of these two time series revealed interesting tide-driven features of lignin concentration. First, they showed that lignin content in particulate matter varies on a daily basis as a function of the tide. This variation was as high as two fold during high flow as compared to low flow in winter, but no changes were seen in the fall sampling. Therefore, the time (ebb vs. flood) and the season of sample collection for lignin analysis is critical when seasonality is to be investigated. That point alone potentially explains that identical variability in lignin measurement was observed in data collected in the winter 1998 time series and throughout the year [Figure 3.6 b)]. This resulted in a less clear-cut seasonality message for land-derived OM contribution to the river than that of autochthonous OM.

The second observation concerns differences in processes that seasonally occur in the river. Fall is a period that follows a low rain, low runoff season, therefore characterized by less land-derived matter input. The lignin content of the SPM is

quite low but constant. During that time high SPM is observed even at low flow owing to autochthonous matter. During high flow the SPM increases as a result of resuspension of sediment mostly composed of minerals. In contrast, winter high flow reveals both a mineral and organic component of land material, while autochthonous OM is not significant. Variation in lignin content depends upon deposition and resuspension of sediments.

A study of the level of decomposition of vascular plant tissues collected during these two time series would have been interesting. However, problems encountered with the use of GFC filters prevented this investigation. Future research should be conducted to determine the daily and maybe monthly variation in SPM lignin content for the four hydrological periods of the Columbia River in order to allow more thorough studies of lignin seasonality.