

CHARACTERISATION OF DISSOLVED ORGANIC CARBON (DOC) LEACHED FROM LEAVES IN WATER

(Karakterisasi Senyawa Organik Karbon Terlarut dari Daun dalam Air)

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ABSTRAK

Penelitian ini bertujuan untuk menentukan senyawa organik khususnya organik karbon terlarut (DOC) dari dua spesies daun tumbuhan (*wandoo eucalyptus* and *pinus radiate, conifer*) yang larut dalam air selama periode 5 bulan leaching eksperimen. Kecepatan melarutnya senyawa organik ditentukan secara kuantitatif dan kualitatif menggunakan kombinasi dari beberapa teknik diantaranya Total Organic Carbon (TOC) analyser, Ultraviolet-Visible (UV-VIS) spektrokopi dan pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS).

Hasil analisis DOC dan UV menunjukkan peningkatan yang tajam dari kelarutan senyawa organik di awal periode pengamatan yang selanjutnya berkurang seiring dengan waktu secara eksponensial. Jumlah relatif senyawa organik yang terlarut tergantung pada luas permukaan, aktifitas mikrobiologi dan jenis sampel tumbuhan (segar atau kering) yang digunakan. Fluktuasi profil DOC dan UV₂₅₄ disebabkan oleh aktifitas mikrobiologi. Diperoleh bahwa daun kering lebih mudah terdegradasi menghasilkan senyawa organik dalam air dibandingkan dengan daun segar. Hasil pyrolysis secara umum menunjukkan bahwa senyawa hidrokarbon aromatic dan fenol (dan turunannya) lebih banyak ditemukan pada residue sampel setelah proses leaching kemungkinan karena adanya senyawa lignin atau aktifitas humifikasi mikrobiologi membuktikan bahwa senyawa-senyawa tersebut merupakan komponen penting dalam proses karakterisasi DOC.

Kata kunci: organik karbon terlarut (DOC), daun tumbuhan, aromatik.

INTRODUCTION

Water contains natural organic matter (NOM) due to the interactions between the hydrological cycle, the biosphere and the geosphere. The large number and complexity of these interactions are responsible for the diverse nature of NOM, with the organic carbon content of a particular water body dependent on the surrounding environment and the biogeochemical cycles associated with it. NOM is a complex mixture of organic material consisting of organics such as, humic acids, hydrophilic acids, proteins, lipids, hydrocarbons and amino acids (Murray and Parsons, 2004). The sources of NOM can be categorized as either allochthonous, entering the system along a transport route from the terrestrial

water shed, or autochthonous, being derived from biota (e.g. algae, bacteria) living within the water-body (Miles, 2005). The term NOM in this report refers to the total dissolved organic matter (DOC) in water derived from terrestrial and aquatic precursor materials.

The presence of natural organic matter (NOM) has been identified as a problem in the supply of safe, potable water. It has the potential to interfere at all stages of the potable water cycle, from the catchment through to the treatment process and then onto the distribution system (Miles, 2005). A study conducted by Murray and Parsons (2004) found that effects of NOM on potable water quality include: (i) enhancement of transport and distribution of organic

micropollutants, (ii) promotion of disinfection by-product (DBP) formation, (iii) undesirable biological growth, and (iv) lowering the efficiency of treatment processes. Accordingly, the health concerns regarding NOM are of similar importance to those concerning heavy metals and other anthropogenic water contaminants.

The measurement of dissolved organic carbon (DOC) is an important part of many water quality studies. The DOC may represent the degradation products of aquatic plant or animal life, or, alternatively, may represent pollution by sewage or industrial effluent. The methods commonly used for DOC determination involve oxidation of the organic material with subsequent quantitative measurements of the CO₂ evolved. Oxidation can be carried out in the gas phase by passing the sample over a catalyst at high temperature or by wet oxidation (Goulden and Brooksbank, 1975). The quantified CO₂ level is then converted to a TOC concentration in the original water sample on the basis of a predetermined standard organic carbon concentration (Issam *et al.*, 2000). The TOC method is a rapid technique that can reliably characterize the organic content of an array of water and waste samples (Kehoe, 1977).

NOM dissolved in water both absorbs light and fluoresces. The absorbance of light is primarily due to the presence of aromatic structures incorporated into the molecules of humic substances. In the UV region, most researchers have limited their data collection to monitoring the absorbance at 254 nm which provides a general indication of overall NOM concentrations. This wavelength also corresponds to the brightest line in the emission spectrum of low-pressure mercury lamps and its absorbance by NOM is usually high enough for reliable measurement. There is a great potential for using absorption spectroscopy to help understand the chemistry of NOM. The technology is not only highly sensitive but practically quite simple to perform. Measurements usually do not require sample preparation, except filtration to remove particles, and the large range of linearity between absorbance and the concentration of NOM measured as DOC typically extends from 0.1 mg/L to several tens or even hundreds of mg/L (Korshin *et al.*, 1996).

Overall, the aim of this project is to examine the magnitude of DOC contribution to

water by leaves materials. The DOC concentration over time is also investigated.

Experimental

Leaves Samples

Three different plant species were sampled from the vegetation surrounding Mundaring Weir Dam. These included *wandoo eucalyptus* and *pinus radiata* (conifer). The fresh and dead leaves of each plant were sampled and stored in plastic bags. In preparation for the leaching experiments, The *corymbia calophylla* leaves were cut in half and the central woody stem was removed.

Milli-Q Water

High purity Milli-Q water was prepared by passing deionised or distilled water through an Elga Milli-Q system. This system in series consisted of both a cation and an anion ion exchange resin column and an activated carbon column, and an ultraviolet sterilization column.

Leachate Experiments

The leachate experiments were all performed with 10 g of plant material immersed in 1.5 L of Milli-Q water, stored in a 2 L glass jar with a lid. The leachates were stirred continuously with a Teflon coated magnetic stirrer bar to improve the stimulation of surface water, as water flow has a significant effect on leaching outcomes (Miles, 2005). Four leachate experiments were carried out and are summarized in Table 1.

Table 1. List of plant materials used in separate leaching experiments.

1. <i>wandoo eucalyptus</i> – fresh leaf
2. <i>wandoo eucalyptus</i> – dead leaf
3. conifer – fresh leaf
4. conifer – dead leaf

Leachate Sampling/Extraction

The leachate was periodically sampled by drawing ~ 15 mL aliquots with a micropipette and was passed through a 0.45 µm filter *via* a glass syringe to isolate the DOC from the particulate organic matter. A very high sampling rate was initially used to capture early leaching behaviour. A daily

rate of sampling was used in the first week but this progressively slowed to weekly, bi-weekly and monthly sampling regimes.

Instrumental Analysis

1. Total Organic Carbon (TOC) Analyser

The dissolved organic carbon concentrations were determined using a Shimadzu TOC-V WS Total Organic Carbon Analyser. All samples were filtered through a 0.45 µm Supor Acrodisc filter prior to analysis. Samples were run in duplicate, and Milli-Q water blanks and standards were frequently run to ensure the instrument was calibrated.

2. Ultraviolet Visible Spectrum Spectrophotometer

UV absorbances at 254 nm of all water samples were measured using a Shimadzu UV-1700 UV/Vis Spectrometer in a 5 cm quartz cell. Background measurements were taken using Milli-Q blanks. All samples were filtered through a 0.45 µm Supor Acrodisc filter prior to analysis.

3. Pyrolysis-Gas Chromatography-Mass Spectrometer (Py-GC-MS)

Dried samples were analysed by pyrolysis-GC-MS on a Hewlett Packard (HP) 5890/5891 GC-MS. Separate analyses were performed on the original and the residual leached plant materials. Each pyrolysis sample (≤ 0.2 mg) was weighed into a quartz capillary tube which was placed inside a coiled filament (platinum) on the pyroprobe which was then inserted into the pyroprobe interface mounted onto the GC injector port.

Results and Discussion

1. Quantitative Assessment of Leaching Experiments

The TOC analyser has been widely accepted for analysis of dissolved organic carbon (DOC). In addition, UV analysis or measurement of absorbance at 254 nm (UV_{254}) has proven to be efficient in identifying aromatic/phenolic organics. The combination of these two techniques provides an indication of the quantitative and some qualitative information about the organic load of source and treated water samples.

The DOC data obtained from the leached fresh and dead leaves of *wandoo eucalyptus* and

conifer species are shown in Figure 3.1. DOC data (Fig. 3.1a) shows a similar pattern to UV absorbance (Fig. 3.1b). Clearly, the DOC shows an initial sharp increase in concentration during the first few weeks of the leaching experiment. It decreases rapidly from day 15, to approx day 20 and then more gradually to day 55 to a plateau reflecting the exponential relationship of leachable organics for the majority of the experiment. The initial rise reflects a significant proportion of organic material being dissolved in the water. The proportion of labile organic matter material decreases with time, until the DOC plateaus from day 55 onwards.

It is apparent that the dead leaf from the *wandoo eucalyptus* showed the highest DOC and UV absorbance and the dead leaf from the conifer showed lowest DOC and UV absorbance. It is also apparent that both the fresh and the dead leaves from the *wandoo eucalyptus* species showed higher DOC and UV absorbance than the corresponding conifer samples.

This may be due to higher surface area of the *wandoo eucalyptus* leaf than conifer needles allowing more material to be leached to the water system. The effect of leaf area on the contribution of aquatic organic compounds is presented by Lockheart *et al.*, (1997) and relates to the intercellular partial pressure of the leaf surface that affects the stomatal conductance to CO_2 . Leaves with higher surface area have more partial pressure resulting in more gases reaching the interior of the leaf and influencing the density of the stomatal pores. When a leaf decays, a leaf with higher surface area leaches higher amounts of aromatic material than a leaf of lower surface area.

Fluctuation pattern of the DOC and UV profiles suggest the occurrence of microbial activity due to rapid loss of bioavailable materials. After the rapid leaching of soluble compounds in the first two weeks of the experiment, more resistant material remains in the leaf residue.

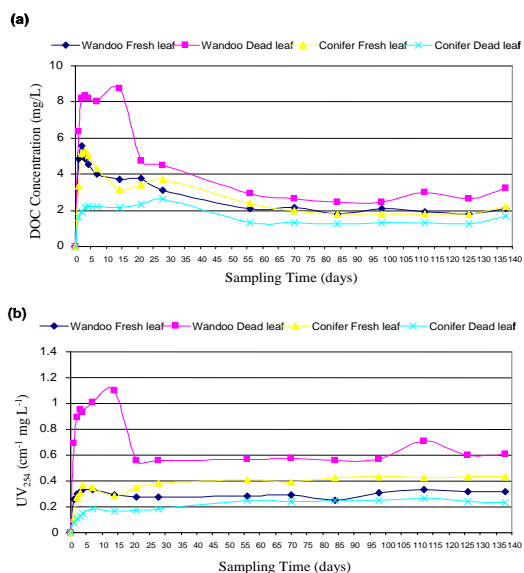


Figure 1. (a) DOC of leaf leachates; (b) UV₂₅₄ of leaf leachates.

A relationship between UV absorbance and the concentration of aromatic hydrocarbons has been previously reported in NOM (Korshin *et al.*, 1996) and is attributed to bacterial precursors. Thus, the drop in UV absorbance may reflect the use of aromatic DOC by bacteria through oxidation, leading to loss of carbon in the form of CO₂ (Veeriah *et al.*, 2004). As previously mentioned, bacterial growth is strongly influenced by water quality, environmental factors and the initial concentration of bacteria. The DOC concentration in Milli-Q water used for these experiments ranged between 0.01 and 0.02 mg/L, thus basic nutrients such as assimilable organic carbon (AOC) present in the water samples plus organic compounds that are derived from the leaching process from plant materials may promote the growth of microbes. Furthermore, aquatic bacteria in the water samples probably consume biodegradable organic carbon (BOC) and also facilitate the hydrolysis of complex organic compounds into AOC, a bioavailable source for bacteria. It is therefore reasonable to assume that both the initial AOC concentration and the concentration of AOC from the leached organic compounds will influence the bacterial growth in the water phase. As a consequence, the rate of bacterial growth is likely to be dependent on the proportion and quality of readily usable/

bioavailable fractions of BOC from leached plant materials.

The results thus suggest that leached organic materials especially within the first 2 weeks of the experiment favour the growth of bacteria. The overall organic content represents both leached organics from the plant materials and the microbial biomass. Interestingly, the bacterial regrowth was more prominent from leaching of *wandoo eucalyptus* leaves compared to the conifer needles.

2. Py-GC-MS Compound Classification

Pyrolysis is a method that thermally cleaves an organic macromolecule into lower molecular weight fragments, some of which can be separated by gas chromatography and identified by mass spectrometry. Under controlled conditions this technique can produce a molecular fragmentation pattern, or fingerprint, which is highly characteristic of the parent organic material (Gray *et al.*, 1996). This method is a powerful tool for characterising biopolymers due to its speed, convenience, and ability to provide complementary information to wet chemical degradation methods.

In order to simplify the interpretation of the data, the identified pyrolysis products were classified into four chemical categories: aromatic hydrocarbons (Aro); phenolic compounds (Ph); carbohydrate derivatives (Cb); and nitrogen-containing products (N-cp). This classification method avoids difficulties associated with attempting more definitive product precursor assignments. Many phenolic and aromatic compounds (e.g. toluene) may be produced from several source biopolymers.

The comparative data shown in Figure 2 reveals that easily biodegradable phenolic/aldehydic aromatics like guaiacol (34) and 4-methylcatechol (46) are completely absent in the residual materials after the leaching period probably due to microbial degradation throughout the leaching period. In contrast, the relative abundance of aromatics including ethylbenzene (7), *m*-xylene (8), styrene (11), and naphthalene (41) were relatively higher in the residual leaf. These may reflect enhanced preservation of the chemical constituents of the leaf, or may include contribution from secondary analytes from the alteration of primary constituents. Several of the most prominent pyrolysis products of the raw *wandoo*

eucalyptus fresh and residue leached leaves are shown in Table 2.

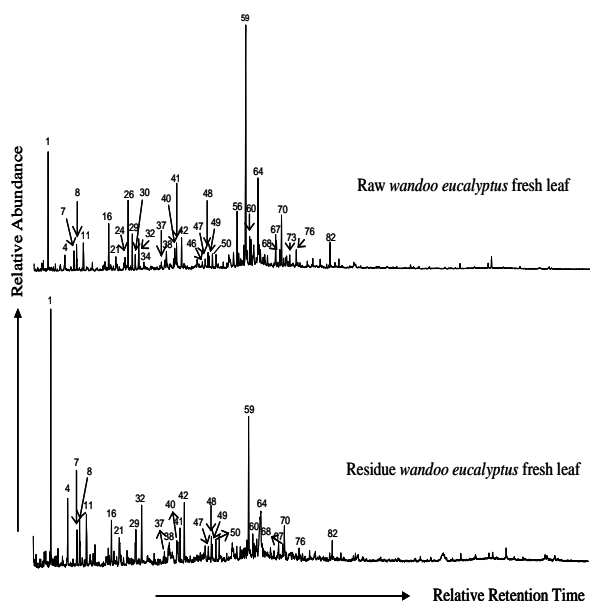


Figure 2. Total ion chromatograms of *wandoo eucalyptus* fresh leaf before (raw) and after (residue) 138 days leaching.

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Table 2. Several abundant pyrolysates identified by Py-GC-MS of the raw *wandoo eucalyptus* fresh leaf and 138 day residue leaf.

Peak No.	Compound	Category	Level of change
4	1,4-Dimethylpyrazole	N-cp	No change
7	Ethylbenzene	ArH	Increase
8	<i>m</i> -Xylene	ArH	Increase
11	Styrene	ArH	Increase
16	5-Methylfurfural	Carbohyd	No change
21	<i>m</i> -Methylstyrene	ArH	No change
32	<i>p</i> -Cresol	Ph-cp	Decrease
34	Guaiacol	Ph-cp	Disappear
41	Naphthalene	ArH	Increase
46	4-Methylcatechol	Ph-cp	Disappear
47	Indole	N-cp	No change
50	2-Methylnaphthalene	ArH	No change

A relatively high abundance of aromatic hydrocarbons has previously been detected in pyrolysis studies of plants (Zhang *et al.*, 1999). Substances such as toluene, styrene, and naphthalene and their alkyl analogues were the main aromatic hydrocarbon compounds from the pyrolysis products of NOM isolated from water and soil (Zhang *et al.*, 1999). Similarly, alkylated benzene and naphthalene were the main aromatic hydrocarbon pyrolysates identified in the present soil analysis. A prominence of phenol and alkylphenol products has also been observed in the pyrolysis of previous water related organic samples (e.g. Lehtonen *et al.*, 2000; Page *et al.*, 2003).

Conclusion

The DOC leached from 2 different plant species sampled from the Mundaring Weir catchment area have been qualitatively and quantitatively assessed using a complimentary array of analytical methods. DOC and UV₂₅₄ analysis measured the DOC concentration of leached plant elements and showed a consistent trend of an initial sharp increase in DOC concentration which then decreases with time to a plateau. The magnitude of the DOC/UV profiles reflect the amount of dissolved organic material. Overall, the DOC and UV data of the leachates and water samples show the dead leaf and flower materials leach significantly more than corresponding fresh materials. The relatively

higher leachability of the dead materials (cf. fresh materials), is probably due to morphological and spatial distribution attributes of epicuticular waxes of surface plant elements (Lockheart, 1997). Epicuticular waxes are largely composed of a mixture of aliphatic and cyclic compounds that form a crystalline structures affecting leaf wettability and influencing transpiration rates. Thus it is reasonable to conclude that dead plant elements are more easily degraded in water systems than fresh elements.

The pyrolysis GC-MS analysis indicated that raw and residue plant materials showed both structural similarities and differences. High abundances of aromatic hydrocarbon and phenolic pyrolysates were consistently detected, likely reflecting their significant contribution to DOC. Several pyrolysates are representative of specific source precursors. For example, lignin derived guaiacol and *m/p*-cresol were very abundant in the pyrolysates of the *corymbia calophylla* bark. Preservation of the *m/p*-cresol products in the bark residue suggests they may derive from lignin moieties of the bark selectively preserved during the leaching process.

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