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Determination of resin acids by gas chromatography and high-performance liquid chromatography in paper mill effluent, river waters and sediments from the upper Derwent Estuary, Tasmania

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ABSTRACT

Resin acids in effluent from a paper mill situated on the upper Derwent Estuary near Hobart, Tasmania (Australia) were determined by HPLC analysis of their 7-methoxycoumarin-4-yl and 7-acetoxycoumarin-4-yl methyl esters. Total concentrations ranged from 1.0 to 4.8 mg l^{-1} with a mean of 2.7 mg l^{-1} during 1991–1992. Capillary GC–flame ionization detection and GC–MS analyses of organic constituents in river waters collected in April 1992 confirmed the presence of resin acids derived from the paper mill effluent, but the concentrations were highly variable and strongly influenced by freshwater flow and tidal movements. At a site just 500 m downstream of the effluent discharge, concentrations ranged from <0.01 to 0.78 mg l^{-1} over a 6-h period. Resin acids were also found in sediments close to the discharge (up to 87 mg kg^{-1} dry mass), but amounts in sediments downriver were generally considerably less (most samples <7 mg kg^{-1}). The major resin acids in the effluent were dehydroabietic, palustric, abietic and pimaric acids. Smaller amounts of isopimaric, neoabietic, levopimaric and sandaracopimaric acids were also found. The proportions of individual resin acids in some of the water and sediment samples showed considerable differences from those in the effluent. The abundance of resin acids with conjugated double bonds such as palustric, levopimaric and neoabietic acids were particularly variable suggesting that they are more easily degraded. Resin acids of the pimarane type, such as pimaric acid, were considerably more stable. Variations in the water column distributions reflect both degradation of the more labile resin acids and redistribution of the resin acids between aqueous, colloid and sediment phases. Dehydroabietic acid was the most resistant to degradation and in some water samples it represented up to 66% of the resin acids compared with only 34% in the effluent. This result confirms earlier observations, and suggests that dehydroabietic acid could be used as a tracer for organic matter derived from the paper mill.

INTRODUCTION

Resin acids are tricyclic diterpenoids which occur naturally in conifers. The major acid is often dehydroabietic acid (DHAA), which has an aromatic C ring (Fig. 1), although which acid predominates depends on tissue type, age and species. Several of the resin acids have two conjugated double bonds, and it is these which appear to be least resistant to chemical degradation. In the manufacturing of pulp and paper, resin acids are released from the wood during chemical and mechanical pulping processes. Large amounts can be present in untreated effluent streams if the mill uses a high proportion of softwood species, such as *Pinus radiata*. This species is fast growing and has good mechanical properties and so it is widely used as a raw material in the Australian paper industry. In *P. radiata*, free resin acids may comprise up to half of the extractable
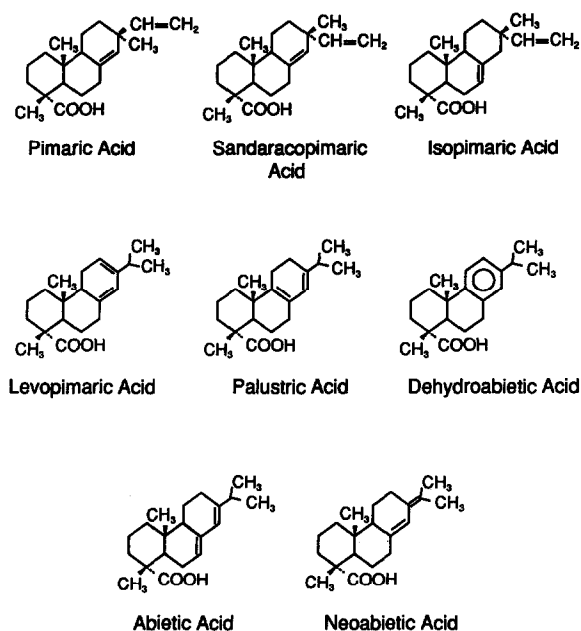
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Fig. 1. Structures of the resin acids referred to in the text.

organic compounds present [1], although the amount present varies with the age of the trees and the conditions under which they are grown [2].

High concentrations of resin acids are acutely toxic to fish so it is desirable to minimise the amounts discharged to the environment. Most resin acids have similar LC_{50} values for fish (0.4–1.7 mg l^{-1}, [3–6]). This range is similar to values obtained for chlorinated guaiacols and catechols in bleached kraft effluent [7–9]. Resin acids are taken up by fish where they concentrate in the liver producing suble-thal effects such as jaundice (accumulation of bilirubin in the plasma) and reduction of UDP-glucuronyl transferase enzyme activity [4,10,11]. In rainbow trout, enzyme activity changes are observed when the fish is exposed to dehydroabietic acid concentrations as low as 0.005 mg l^{-1} [10], although Oikari et al. [4] suggested that 0.02 mg l^{-1} is close to the "minimum effective concentration" of dehydroabietic acid to rainbow trout. At 0.4 mg l^{-1} or higher concentrations, rainbow trout develop jaundice in 2–4 days at water temperatures over 17°C, but they can recover from severe intoxication within 6 days [11].

Over recent years, there has been considerable concern about the water quality of the Derwent Es-

tuary in Tasmania due to the impact of industry and sewage effluent. The condition of the upper estuary has been affected by the input of wood fibre and extractives into the river from the Australian Newsprint Mills over a 45-year period [12–14]. Large deposits (estimated to exceed 4 million m^3 in 1988) of organic-rich sludge have been found on the river bed downstream of the mill [14]. The study described here was carried out using samples collected in March 1990 and April 1992 to assess the concentrations of resin acids in the waters and sediments near to, and downstream of, the mill as part of a larger study to assess environmental conditions in the Derwent river and estuary.

EXPERIMENTAL

Collection and extraction of water and effluent samples

Samples of effluent from the paper mill were collected throughout 1989–1992 at approximately 14-day intervals from a sampling site in the effluent stream just prior to its discharge into the river. Each sample was a composite of effluent collected every hour over the 24-h period.

Surface river water samples were collected on April 1, 1992 in pre-cleaned Niskin bottles over a depth of 0.5–1.5 m. A 500-ml subsample from the 10 l collected by each Niskin bottle was immediately placed into glass bottles for transport to the laboratory. Resin acids have a strong affinity for glassware and plastics so it was essential that all equipment was cleaned thoroughly before use. All glassware was washed in 1% Extran solution, rinsed in Milli Q water, and then rinsed in Nanograde acetone before use. Resin acids remain in alkaline solution, so the 500-ml water samples were immediately adjusted to pH 11.0 by adding KOH. This procedure also liberates resin acids bound to particles in the water so the method gives total dissolved plus particulate resin acids.

The extraction method used here followed that of Richardson et al. [15]. In the laboratory, the water was filtered through a glass fibre Whatman GF/C filter to remove particulate matter such as wood fibre. The conductivity of the filtrate was adjusted to > 2.0 mS cm^{-1} by adding solid NaCl and the pH was adjusted to 8.4–8.6 immediately before extraction. The water sample was then placed on a C_{18}

Bond Elut column under slight vacuum at a constant flow of approximately 1.0 ml min^{-1}. The Bond Elut column had been prepared by washing with acetone (2 × 1 ml), methanol and distilled water. Failure to maintain a constant ionic strength resulted in low and variable recoveries of the resin acids from the Bond Elut column. The vacuum was left on for 5.0 min after the sample had run through to remove any water remaining in the column. The resin acids were eluted with two 1-ml washes of acetone, dichloromethane, and methanol. This differs slightly from the procedure used previously by Richardson et al. [15]. Heptadecanoic acid methyl ester (17:0) was added to the combined eluents as an internal standard, and the samples were then dried under nitrogen. Resin acids were converted to methyl esters using diazomethane. Other esterification reagents, such as 14% BF$_3$ in methanol, were tried, but all gave low recoveries of esterified resin acids.

Sediments

Sediments were collected using a Smith-MacIntyre grab sampler at the same sites as the river water samples above, adjacent and downstream of the mill. Representative subsamples of the sediments were stored in glass jars and refrigerated until analysed. A 60-g portion of the wet sediment was freeze dried, and 10 to 15 g of the dried sediment was then extracted with 200 ml of acetone. The sediment-solvent mixture was agitated at hourly intervals for 5 h and then left to stand overnight. The acetone was then decanted and fresh solvent added (2 × 25 ml) to remove any residual resin acids. The combined extracts were filtered through a 0.2-μm Anatop disposable filter to remove any sediment particles, rotary evaporated to dryness and derivatized with diazomethane as previously described. Each sample was analysed by capillary gas chromatography after addition of the 17:0 methyl ester internal standard.

The presence of high loadings of organic matter in the sediment extracts produced very complex chromatograms so it was necessary to purify the extracts further. The total extract was dried under nitrogen, then dissolved in Milli Q water at pH 11.0 (KOH) and loaded onto a Bond Elut C$_{18}$ column prepared as for the water extraction. The resin acids were eluted with two 1-ml portions of acetone, derivatised and then analysed as before. This procedure removed a number of compounds which interfered with the analysis, but resin acids were quantitatively recovered unchanged.

Quantification of resin acids in effluent using high-performance liquid chromatography

The concentration of total resin acids in effluent was monitored approximately every two weeks using the HPLC method described by Richardson et al. [15]. Briefly, the resin acids were extracted by passage through a C$_{18}$ cartridge at pH 9 and converted to 7-methoxycoumarin-4-yl methyl esters (MMC) and 7-acetoxycoumarin-4-yl methyl esters (MAC) of the resin acids using 4-bromomethyl-7-methoxycoumarin and 4-bromomethyl-7-acetoxycoumarin respectively. The HPLC analysis used a Rainin Dynamax C$_8$ 5-μm 25 cm × 4 mm I.D. reversed-phase column with guard column and filter. The solvent system was acetonitrile–water (70:30, v/v) at 1.5 ml min^{-1} followed by a linear gradient to acetonitrile–water (90:10, v/v) at 1.5 ml min^{-1}. The MMC esters were detected by UV absorption at 318 nm while the 7-hydroxycoumarin-4-yl methyl esters obtained by post-column alkaline hydrolysis of the MAC esters [15] were detected using fluorescence. The detection limits were 0.02 mg l^{-1} and 0.001 mg l^{-1}, respectively. The resulting chromatograms show a peak for dehydroabietic acid and a single, later-eluting peak for all other non-aromatic resin acids. Monocarboxylic fatty acids produce an additional peak or peaks later in the chromatogram (Fig. 2).

Identification of resin acids by capillary GC with flame ionization detection (FID) and GC–mass spectrometry

Resin acid methyl esters were analysed by capillary GC using a HP-1 methyl silicone non-polar capillary column (50 m × 0.32 mm I.D., 0.17 μm film thickness). The samples were injected using a cooled OCI-3 on-column injector and the constituents were detected with a flame ionization detector operated at 310°C. A column temperature program of 45 to 140° at 30°C min^{-1}, and 140 to 310°C at 4°C min^{-1} was used. Resin acids were identified by comparing retention time data with those of laboratory standards and from mass-spectral data obtained from a Hewlett-Packard 5970 MSD coupled to an HP 5890 GC by a direct capillary inlet. The
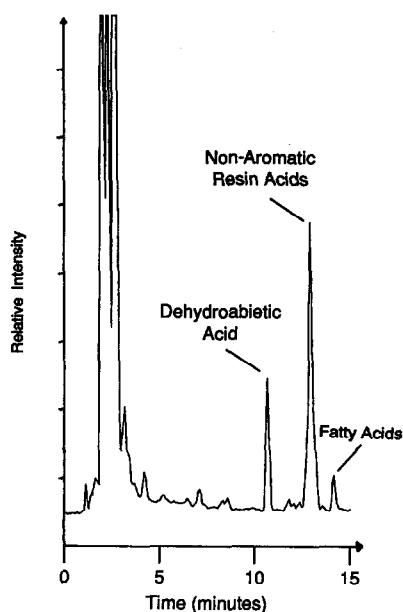
Fig. 2. Typical HPLC chromatogram showing resin and fatty acid constituents in effluent from the paper mill. The main peaks in increasing time of elution are: dehydroabietic acid, non-aromatic resin acids and monocarboxylic fatty acids, all as (methoxycoumarin-4-yl) methyl esters.

non-polar column, injector and chromatography conditions were similar to those described above with the exception that helium was used as the carrier gas. Electron impact mass spectra were acquired and processed with an HP 59970A Computer Workstation. Typical MSD operating conditions were: electron multiplier 2200 V; transfer line 310°C; electron impact energy of 70 eV; 0.8 scans s; mass range 40–650 u. Recoveries and response factors were determined by analysing known amounts of resin acid standards spiked into water and sediment samples.

RESULTS AND DISCUSSION

Comparison of the HPLC and GC–MS methods

The methods used in this study were designed to be applicable for routine monitoring and for more detailed characterization of organic constituents in effluents, process streams, river waters and sediments. The HPLC method (Fig. 2) was ideal for monitoring total concentrations of resin acids and

dehydroabietic acid in effluent and in-plant aqueous streams [15]. The GC–MS method was more time consuming both in instrument time and analysis of the data, but it provided a better appreciation of changes in composition of the resin acids in the sediments and waters of the estuary. Richardson et al. [15] have previously shown that results from both the GC and HPLC methods show excellent agreement. The HPLC method with solid-phase extraction can be used at concentrations of resin acids in water at concentrations as low as 0.001 mg l^{-1} [15], although information about individual resin acid abundances is not obtained apart from dehydroabietic acid.

Several papers describe the analysis of resin acids in effluents [9,15,16]. Concentrations are often very high (of the order of several mg l^{-1}) and so the methods used do not require the same high sensitivity as those required for analysis of natural waters. Kal'chenko and Svitel'sky [16] reported that chloroform–diethyl ether can be used to extract a variety of constituents including resin acids, fatty acids and phenols in paper mill effluents and white waters although recovery figures were not given. Lee et al. [9] used methyl tert.-butyl ether followed by transfer to acetone which yielded recoveries generally greater than 95%, except at concentrations of resin acids <0.01 mg l^{-1}. These authors remarked that dichloromethane can cause emulsions with effluent samples, and that hexane gave recoveries of only 60% or less.

There are still surprisingly few studies of resin acids in matrices other than water such as sediments. Some early work using packed GC columns was reported by Brownlee and co-workers [17,18]. More recently, a very sensitive method based on the analysis of pentafluorobenzyl esters by capillary GC with ECD detection and negative-ion chemical ionization mass spectrometry has been developed [19,20], but we chose to base our methods on HPLC, capillary GC and GC–MS equipment that would be readily available in most analytical laboratories. Morales et al. [21] recently reported methods for the analysis of resin acids in water, sediments and fish bile based on extraction from a pH 5 medium with methyl tert.-butyl ether–dichloromethane solutions.

The non-polar GC column provides very good separation of the methyl esters of resin acids from

other components such as fatty acid methyl esters, but levopimaric and palustric acids which have very similar structures (Fig. 1) are not separated. The two have quite different mass spectra, so GC–MS data (e.g. selected ion monitoring [21]) can be used to quantify them if required. The major ions in methyl levopimarate in decreasing order of abundance are: m/z 91 (base), 121, 146, 316 (molecular ion), 187, 256 and 241 (weak). In contrast, methyl palustrate has a base peak at m/z 301 (not found in methyl levopimarate) and major ions at m/z 241 and 316. For example, GC–MS data confirmed that levopimaric acid was a minor constituent of this peak in chromatograms of the resin acids in the effluent, and water samples near to it, but in sediment samples the peak was almost entirely due to palustric acid methyl ester. Mass spectra of common resin acid methyl esters are given in ref. 22, and characteristic ions for resin acid methyl esters and some chlorinated products are given in ref. 21. DB-5 and DB-17 GC column phases also provide useful separations of the pentafluorobenzyl esters of most of the common resin acids [9,19].

The recovery of resin acids in effluent using the HPLC procedure was 95 ± 8% for dehydroabietic acid and 91 ± 11% for the non-aromatic resin acids [15]. Recoveries of spiked samples using the GC and GC–MS procedure for the water and sediment samples (where concentrations were much less) were greater than 75%. In contrast, Lee and Peart [19] found that soxhlet extraction of resin acids from sediments with acetone was 10–30% more efficient than either a high-speed homogenizer or ultrasonic extraction. The efficiency was further improved (by 200–300%) using acidified 12% methanol in acetone, except for palustric and neoabietic acids which isomerize under acid conditions. More recently, these same authors [20] showed that extraction with supercritical carbon dioxide from a 1:1 mixture of methanol and formic acid yielded quantitative recoveries of most resin acids, apart from palustric and neoabietic acids which were recovered in 40% yield [20]. Our experience also shows that the widely used Bligh and Dyer technique using chloroform–methanol mixtures gives very poor yields [14].

Composition and concentration of resin acids in effluent

Total concentrations of resin acids in the paper mill effluent over the 12 months from March 1991 to March 1992 determined by the HPLC method ranged from 1.0 to 4.8 mg l^{-1}, with an average of 2.7 mg ml^{-1}. Routine monitoring of the effluent by the paper mill in 1989 showed that the mean concentration of resin acids was 3.5 mg l^{-1}. These variations mainly reflect day to day changes to operations in the paper mill and differences in the amounts of paper produced each month, although some improvement in effluent water quality from 1989 to 1992 is apparent. Process waste waters within the paper mill consist of brown water (mainly dissolved and colloidal organic matter) and white water (mainly suspended fine pine fibre) streams each of which receives separate primary treatment. Alum is added to the white water clarifier at a concentration of 60 mg l^{-1} which removes a substantial fraction of the resin acids associated with fibre and particles. The concentration of dehydroabietic acid is reduced by about 70% compared with the input stream and other resin acids are reduced by about 80%. However, the addition of alum to the brown water stream is not effective in removing resin acids presumably because they are mainly associated with colloidal organic matter. Even without the addition of alum, reductions of 10 and 27% in dehydroabietic and non-aromatic resin acid abundances in the brown water clarifier are still achieved. The effluent concentrations found here are typical of older mills lacking secondary treatment (e.g. 3.4 mg l^{-1} [18]). Much lower effluent concentrations of 0.058, 0.17 and 0.68 mg l^{-1} were recently reported by Lee et al. [9] for three Canadian pulpmill effluents. Variations in effluent concentrations reflect different conifer feedstocks and the use of more efficient secondary treatment of the effluent.

The major resin acids in the effluent collected at the same time as the water and sediment samples were dehydroabietic, palustric, abietic and pimaric acids. Smaller amounts of isopimaric, neoabietic and sandaracopimaric acids were also found. Similar distributions are found throughout the year. These resin acids are all common constituents of conifers, and similar distributions have been reported for Canadian pulpmill effluents [9]. Comparisons of the relative abundances found with those in the

softwood feedstocks indicate that changes and losses of resin acids occur during the paper making process. For example, levopimaric and palustric acids together represent about 40–45% of the total resin acids immediately after pulping, but this value is reduced to less than 20% in the final effluent (unpublished data).

Resin acids in surface waters of the Derwent estuary

The mill presently discharges 70–80 million l of effluent per day into a river flow of about 4000 million l per day. The effluent forms a well-defined tannin-coloured plume for at least 1 km downstream of the mill, but the location of the plume in the river and the extent of its mixing with the freshwater flow is quite variable.

The control site for the 1990 survey was situated approximately 12 km upstream of the mill. There are no industrial inputs above this point in the river, although small chemical and nutrient inputs from agricultural activities would be expected. The bottom salt wedge often extends past the paper mill during low river flows in summer, but it does not extend past New Norfolk 5 km upstream of the mill and thus we would not expect to find resin acids indicative of the mill effluent to be present at the control site. Resin acids were not detected in the water column at the 1990 control site which is consistent with the predominance of eucalypts —which do not contain resin acids— in the catchment area and sparse cover of conifers. This result indicated that resin acids are at most trace constituents in the water used by the paper mill.

In the 1992 study we moved the control site (site A) to approximately 2 km upstream from the paper mill outfall. Saline water is carried upstream of the mill as a near-bottom salt water wedge during periods of high tide and low river flow, but judging from the very low concentrations of resin acids detected in waters and sediments at this site the transport of contaminants upstream is relatively minor. Of the resin acids detected, dehydroabietic and pimaric acids were most abundant, but the abundances of resin acids with conjugated double bonds were very low or not detectable.

Gas chromatograms of resin acids in the effluent and river water samples within 6 km of the mill are quite similar apart from additional compounds present in the river water samples and small variations in resin acid abundances. The total concentrations of resin acids in river water at sites downstream of the paper mill during the 1992 study are shown in Table I. At site B, which is directly in front of the effluent discharge, the concentration of total resin acids was 0.19 mg l^{-1}. In the 1990 survey, the corresponding value was very similar (0.21 mg l^{-1}) which is approximately 14 fold less than typical concentration of 2.7 mg l^{-1} in the effluent. The distribution of resin acids was quite similar to that in the effluent sample collected on the same day (Table I), and the differences were within the range of variations found in effluent composition (unpublished data).

The concentration of resin acids in the water column at site C, 500 m downriver, was much less at only 0.018 mg l^{-1} suggesting that this sample was collected on the edge of the plume which was not well defined on the day of sampling. In 1990, the corresponding concentration at this site was 0.15 mg l^{-1} but, as we show later, such extreme variability can be caused by the state of the tide changing the position of the effluent plume in the river. Surface water concentrations further downstream at site I, 11.6 km from the mill, were even lower at 0.008 mg l^{-1}.

Fox [23] carried out a study of resin acids in waters of Lake Superior which received effluent from a kraft pulp mill. The concentration of dehydroabietic acid was 1.93 mg l^{-1} at 300 m from the effluent (c.f. 1.5 mg l^{-1} in the effluent in our study) and decreased to 0.018 mg l^{-1} at 1.7 km from the mill which is comparable to most of the downriver sites analysed here from the Derwent estuary. In the Lake Superior study, organic constituents in the effluent were reduced to background levels within the first 2 km due to efficient mixing processes, but in the Derwent they can still be detected in waters 10 km downriver (albeit in low concentration). Based on the volume of effluent discharge compared with the river flow, if there were complete mixing this would produce resin acid concentrations of 0.05–0.06 mg l^{-1} which is considerably in excess of most of the concentrations measured (0.008–0.04 mg l^{-1} at sites 500 or more meters from the effluent; Table I). Although the plume is well defined near the mill, one would expect that the waters would be reasonably mixed at sites more than 5 km from the mill such as G, H and I. The lower than expected con-

TABLE I

PERCENTAGE COMPOSITION AND TOTAL CONCENTRATION OF RESIN ACIDS IN PAPER MILL EFFLUENT AND SURFACE WATER SAMPLES FROM THE UPPER DERWENT ESTUARY

All samples were collected on April 1, 1992. Site A is upstream of the mill, and site B is immediately adjacent to the mill outfall. All other sites are downriver.

Resin acids	Effluent	Sites (distance from mill in km)							
		A (−2.1)	B (0)	C$_4$ (0.5)	E (3.6)	F (4.7)	G (5.8)	H (8.6)	I (11.6)
Pimaric	11.6	23.8	15.2	16.0	17.1	13.9	15.2	17.5	12.4
Sandaracopimaric	2.2	2.4	2.3	3.3	2.9	2.5	0.8	0.6	2.4
Isopimaric	10.6	9.0	10.3	10.0	10.3	11.1	12.7	10.7	10.8
Levopimaric + Palustric	18.9	0.0	16.4	0.3	0.4	11.4	8.3	4.9	9.2
Dehydroabietic	33.8	63.3	33.8	67.0	67.0	42.7	50.1	63.9	45.2
Abietic	14.9	1.5	17.1	2.1	1.8	17.4	12.3	2.4	17.4
Neoabietic	8.1	0.0	5.0	1.3	0.4	1.8	0.7	tr^a	2.6
Total %	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Conc. mg l^{-1}	4.5	0.0023	0.192	0.0186	0.0312	0.0395	0.0172	0.0192	0.0084

^{a} tr = trace; <0.1%.

centrations in the surface fresh water imply that a major part (perhaps about 50%) of the resin acid load is transferred to the underlying salt wedge and bottom sediments.

The changes in proportions of individual resin acids at the different sites cannot be explained in terms of a simple model of effluent mixing with the river water. At sites C and E, the proportions of individual resin acids were markedly different from those in the effluent with abietic, levopimaric, palustric and neoabietic acids present in much reduced amounts (Table I). This distribution more closely resembles that found at the upstream control site A, although the total concentrations were 15–20 times lower there. Compositional changes were also apparent at sites further downriver, but a regular trend was not apparent.

Although most resin acids are rapidly degraded by microorganisms in aquatic environments [24], dehydroabietic acid appears to be quite persistent [23] with an apparent half-life in water of about 0.12 years and in sediments of 21 years [17]. It seems unlikely that the rapid transport of resin acids downriver within the fresh water phase would allow enough time for microbial processes to change the resin acid proportions significantly. It are only those resin acids which contain conjugated double bonds (mainly of the abietane class) which show

major changes in proportions. These are the same acids which are degraded and interconverted during paper pulping and processing suggesting that chemical processes are responsible. Since much of the resin acids in the effluent are associated with colloids, the sedimentation of this material to the sediment–water interface could account for the reduced concentrations found in the fresh water phase. Also, since these colloids and bottom sediments are readily resuspended this process could reinject the resin acids back into the water column, but over a longer time frame which is sufficient to allow for chemical modification of the resin acid distributions to take place.

Effects of tidal mixing on water column concentrations of resin acids

The concentrations of resin acids in surface waters of the estuary are strongly influenced by the mixing of fresh and salt waters, and by the effects of the tide. To assess this, water samples were collected at 4 times during the day from 4 stations across the river at site C, 500 m downstream of the effluent. An additional sample was collected from site F* near to Green Island 5 km downstream of the mill. Data were obtained on October 29, 1990; February 29, 1991; June 3, 1991; September 30, 1991; and on January 13, 1992. Data from the latter sampling,

which was closest in time to the river survey, are shown in Table II. It should be noted, however, that the concentration data from the other sampling dates showed considerable variation.

Samples collected at 08.35 in the morning showed very low concentrations of resin acids (most <0.01 mg l^{-1}) at all sites across the river, but two h later at 10.35 the concentrations had increased to 0.18 mg l^{-1} except near the inner bank where the concentration was still <0.01 mg l^{-1}. Concentrations were even higher after mid-day and reached maximum values of 0.78 mg l^{-1} in the early afternoon due to the incoming tide pushing the effluent stream to the far bank. The variations at site F* near Green Island are less affected by the tide, but even here the concentrations ranged from <0.01 to 0.05 mg l^{-1} which might indicate incomplete mixing of the plume (at least on some occasions). It is clear from these data that any attempt to model the transport of resin acids in the estuary must include the effects of water mass mixing due to currents and tides. Discrete water samples can only provide a snapshot of contaminant loads in the river and estuary and integrated samples which distinguish between the surface fresh waters and deeper waters of the salt water wedge are essential to obtain a better budget of contaminant loads and transport in the estuary.

Resin acids in sediments

Resin acids distributions in sediments collected on April 1st, 1992 are shown in Table III. A representative chromatogram of the resin acids in sediment from site G is shown in Fig. 3. Note that although this extract had been purified by passing it through a Bond Elut cartridge, the chromatogram still shows many peaks due to compounds other than resin acids. Straight-chain, branched-chain and unsaturated fatty acids account for many of the peaks present, but many unidentified compounds are also abundant.

Peaks due to fatty acids are of similar abundance to those for the resin acids, but this underestimates their true concentration since the extraction method is not designed for quantitative recovery of fatty acids. In another study [13] we showed using extraction with chloroform–methanol that the concentration of fatty acids in fibre-rich sludges from the Derwent estuary were as high as 2300 mg kg^{-1} compared with a background figure of about 50 mg kg^{-1} in deeper sediments deposited before the paper mill was operational. Note that the bacterially derived C_{15} iso- and anteiso-branched fatty acids are as abundant as the C_{16} and C_{18} fatty acids from wood fibre and higher plants testifying to the high bacterial biomass and intense anaerobic metabolism and breakdown of organic matter occurring in the sediments.

At site A 2.1 km upstream of the paper mill, some resin acids were detected in the sediment, but the amounts were extremely low (0.34 mg kg^{-1}). The salt wedge at the time of sampling extended well past site A almost to the New Norfolk bridge and

TABLE II

CONCENTRATIONS (mg l^{-1}) OF TOTAL RESIN ACIDS, AMOUNT OF NON-FILTERABLE RESIDUE (NFR), pH AND CONDUCTIVITY IN SURFACE WATERS FROM TWO SITES IN THE UPPER DERWENT ESTUARY DOWNSTREAM OF THE MILL COLLECTED AT 2-h INTERVALS ON JANUARY 13, 1992

Site[a] number	Time	Total resin acids	NFR[b]	pH	Conductivity
C_1	08.35	<0.01	1.2	7.3	10.4
C_2	08.35	0.02	3.6	7.6	9.6
C_3	08.35	<0.01	2.8	7.7	9.6
C_4	08.35	<0.01	2.8	7.6	9.9
F*	08.35	<0.01	1.8	7.5	14.3
C_1	10.35	0.17	3.4	7.5	10.3
C_2	10.35	0.07	2.0	7.5	11.3
C_3	10.35	0.18	3.8	7.6	11.0
C_4	10.35	<0.01	1.4	7.6	11.3
F*	10.35	0.02	2.0	7.5	11.1
C_1	12.40	0.29	6.0	7.8	12.8
C_2	12.40	0.21	3.6	7.7	12.6
C_3	12.40	0.15	2.6	7.5	12.6
C_4	12.40	0.07	2.0	7.5	13.1
F*	12.40	0.02	0.6	7.5	10.8
C_1	14.35	0.78	13.8	8.2	13.2
C_2	14.35	0.42	6.4	7.8	13.1
C_3	14.35	0.08	2.4	7.5	13.1
C_4	14.35	0.07	2.8	7.5	13.0
F*	14.35	0.05	1.8	7.3	11.6

[a] Site C is 500 m.

[b] NFR is non-filterable residue which contains a large contribution of wood fibre from the paper mill effluent. downstream: samples 1–4 were collected across the river with sample 1 on the opposite bank to the mill. Site F* is on the north end of Green Island 5 km from the mill, slightly downriver from site F in the 1992 study.

TABLE III

PERCENTAGE COMPOSITION AND TOTAL CONCENTRATION OF RESIN ACIDS IN SEDIMENTS FROM THE UPPER DERWENT ESTUARY

All samples were collected on April 1, 1992. Site A is upstream of the mill, and site B is immediately adjacent the mill outfall. All other sites are downriver.

| Resin acids	Sites (distance from mill in km)								
	A (−2.1)	B (0)	C_4 (0.5)	D (1.5)	E (3.6)	F (4.7)	G (5.8)	H (8.6)	I (11.6)
Pimaric	9.0	7.5	14.9	13.7	30.3	9.3	9.5	9.1	8.5
Sandaracopimaric	0.7	0.7	5.3	1.6	3.1	5.5	3.3	3.0	3.8
Isopimaric	8.6	13.4	19.2	20.3	13.2	8.8	15.3	16.2	20.7
Levopimaric + Palustric	0.0	0.5	0.4	2.7	5.6	5.1	2.3	2.7	3.7
Dehydroabietic	80.1	62.0	38.3	43.4	29.2	51.2	49.4	51.7	51.4
Abietic	1.7	15.1	19.0	15.2	16.0	11.0	11.1	11.7	9.6
Neoabietic	0.0	0.8	2.9	3.1	2.6	9.1	9.1	5.5	2.3
Total %	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Concentration mg kg^{-1} (dry wt.)	0.34	87	1.2	34	0.7	7.0	5.3	4.0	1.5
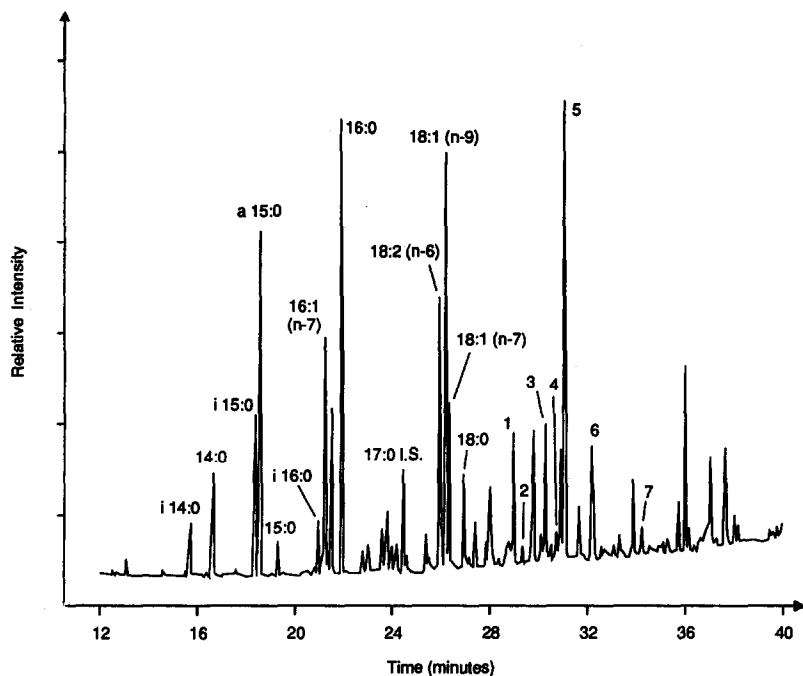
Fig. 3. Partial capillary GC–FID chromatogram of resin acids in sediment from site G. Peaks are methyl esters of (1) pimaric, (2) sandaracopimaric, (3) isopimaric, (4) levopimaric plus palustric, (5) dehydroabietic, (6) abietic and (7) neoabietic resin acids. Heptade-canoic acid methyl ester (17:0) was added as an internal standard (I.S.). Fatty acids are designated number of carbon atoms: number of double bonds. Branched fatty acids are indicated by iso (i) and anteiso (a). The group of compounds eluting after the resin acids was not identified.

this would have carried upstream a small amount of organic material from the effluent. Indeed, analysis of a saline bottom water sample from just below the outfall showed concentrations of resin acids approximately half those in the overlying fresh water (unpublished data). Dehydroabietic and pimaric acids dominated the distribution at site A (Table III), indicating that these resin acids are the most stable of those studied. The absence of the resin acids with conjugated double bonds such as levopimaric and palustric acids confirms that these resin acids are readily degraded.

At site B close to the discharge point, the total resin acid concentration in the sediment was 87 mg kg^{-1} (dry wt.). At site C_4 500 m downriver the concentrations of resin acids was only 1.2 mg kg^{-1} and yet at site D the concentrations were considerably higher at 34 mg kg^{-1}. This extreme variation is largely due to the changing composition of the bottom sediments. At site C_4 the sediment was very sandy and there was little evidence of wood fibre present. In contrast, at site D where the concentration of resin acids was higher, there has been extensive deposition of wood fibre around the inner bend. These sediments contained considerable silt and organic matter and they were strongly anoxic reflecting substantial microbial reworking of the organic matter present.

The presence of resin acids in water at sites H and I, which are over 10 km from the mill, is difficult to explain simply due to the water transport of dissolved constituents. The river is only 3 m deep at these sites in this part of the estuary (apart from a narrow central channel) and the river flow at the edges is much slower leading to considerable sediment build-up at sites H and I. These sediments still contain resin acids and other organic compounds from the paper mill, and these sediments can be resuspended especially during periods of high freshwater flow leading to higher than expected water column concentrations.

CONCLUSIONS

GC–FID, GC–MS and HPLC methods have been developed to study resin acids in effluents, river waters and sediments. The HPLC method provides concentration data for dehydroabietic acid and total non-aromatic resin acids. It is less time consuming than the GC–MS determinations and thus it is ideal for routine monitoring of total resin acid concentrations. The GC–MS method is better suited to environmental applications such as water and sediment analyses where concentration data are required for individual resin acids. Surface waters and river sediments from the upper Derwent estuary contain resin acids derived from paper mill effluent. The distribution of resin acids in the rivers shows considerably more variation than is found in the effluent providing evidence that the resin acids are degraded and remobilised in the estuary. The resin acid concentrations are much less than those which have been found to cause mortality in fish, but they are only just below the levels found to cause sublethal effects. A more extensive monitoring program would be needed to determine the three-dimensional transport of effluent constituents in the estuary and the relative importance of sediment resuspension as a contributing source of contaminants in the water column.

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