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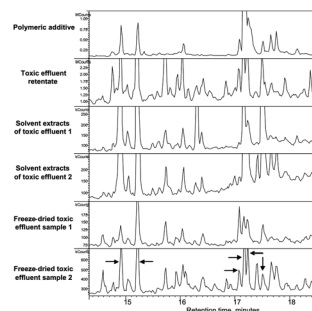### Using Py-GC/MS to fingerprint additives associated with paper mill effluent toxicity episodes

B. Bruce Sithole,* Jorge Pimentel, Sharon Gibbons and Chu Watanabe

Understanding the cause of effluent toxicity is an important requirement for its prevention, remediation and return to compliance.

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PAPER

Using Py-GC/MS to fingerprint additives associated with paper mill effluent toxicity episodes

B. Bruce Sithole,*[a] Jorge Pimentel,[b] Sharon Gibbons[b] and Chu Watanabe[c]

Understanding the cause of effluent toxicity is an important requirement for its prevention, remediation and return to compliance. One component of the strategy entails identification and fingerprinting of additives or components in additives that may be the cause of the toxicity episodes. A number of additives used in pulp and papermaking are polymeric compounds that are suspect in effluent toxicity. Their analysis and detection is difficult as they are not amenable to analysis by normal techniques applicable to mill effluents such as gas chromatography. Py-GC/MS is a powerful analytical technique that can be used to fingerprint these additives. The presence of the additives is confirmed by fingerprint pyrograms of the additives (or components in the formulations of the additives) in conjunction with mass spectrometry. The technique has been used to fingerprint and quantify polymeric additives associated with mill effluent toxicity episodes.

Introduction

Effluents discharged from pulp and paper mills are regulated for toxicity to aquatic biota: the regulation mandates ≥50% survival of rainbow trout (*Oncorhyncus mykiss*) and *Daphnia magna* exposed to full-strength effluent in 96 h and 48 h toxicity tests, respectively.[1] The tests with rainbow trout must be done once a month, whereas the tests for *Daphnia magna* must be done weekly. When an effluent fails to meet the regulatory limit, the frequency of the testing must be increased and continued until three consecutive samples, taken on different days, met the regulatory limit.[1]

Understanding the cause of effluent toxicity is an important requirement for its prevention, remediation and return to compliance. The US Environmental Protection Agency has issued protocols for toxicity identification evaluation that are generic and not streamlined for any particular industrial effluent.[2–4] The protocols are sufficiently complex that they are difficult to implement in mills. Consequently, we have developed an industry-specific diagnostic strategy that can be used by mill staff to help troubleshoot the most common cause of toxicity episodes and thus facilitate remedial steps for retuning to compliance.[5] Potential components in mill effluent components that can cause toxicity include: natural constituents of wood (wood lipophilic extractives); substances formed during

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Environmental impact

Understanding the cause of effluent toxicity is an important requirement for its prevention, remediation and return to compliance. The US Environmental Protection Agency has issued protocols for toxicity identification evaluation that are generic and not streamlined for any particular industrial effluent. The protocols are sufficiently complex that they are difficult to implement in mills. Consequently, we have developed an industry-specific diagnostic strategy that can be used by mill staff to help troubleshoot the most common cause of toxicity episodes and thus facilitate remedial steps for retuning to compliance. Potential components in mill effluent components that can cause toxicity include: natural constituents of wood (wood lipophilic extractives); substances formed during pulping or bleaching (process derivatives); additives (slimicides, dyes, surfactants, *etc.*); and effluent treatment related factors (ammonia, carbon dioxide, pathogens, *etc.*). However, in many instances, identifications of the causes of the toxicity could not be traced to wood lipophilic compounds or effluent treatment related factors. The only other likely option was additives used at the mills. Extensive tests have shown that additives used in the pulp and papermaking process can contribute to mill effluent toxicity. Consequently, one component of the strategy entails identification and fingerprinting of additives or components in additives that may be the cause of the toxicity episodes.

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A number of additives used in pulp and papermaking are polymeric compounds that are not amenable to analysis by normal techniques applicable to mill effluents such as gas chromatography. These polymers are best analysed by analytical pyrolysis. In this technique, thermal energy is applied to the compounds to break them into products that are volatile enough to be analysed by gas chromatography.[7] The pyrolysis is done under controlled conditions and inert atmospheres such that the resultant pyrolysis fragments give characteristic patterns that are "fingerprints" of the original polymers. Further information on the chemistry of the fragments can be obtained by detecting them by mass spectrometry. This technique is called pyrolysis gas chromatography with mass spectrometric detection (Py-GC/MS). In this report, we describe applications of this technique in fingerprinting additives associated with mill effluent toxicity episodes.

A mill conducted a trial on a new retention aid that had been introduced into the market. Unfortunately, there was a spill and a large quantity of the product was discharged to the mill effluent system. Soon after this, the mill experienced on-going effluent toxicity episodes and was not able to meet the regulatory target in tests with *Daphnia magna*. Estimates were that the spill contributed 1.9 mg L^{-1} of the polymer in the final effluent. Toxicity testing of the polymer showed that the LC50 for *Daphnia magna* was 1.4 mg L^{-1} indicating that the polymer was likely present at toxic levels in the mill effluent. Unfortunately, conventional analytical techniques could not confirm its presence. Subsequent to the original spill, toxicity episodes occurred sporadically over a 2 year period. Toxicity identification evaluation (TIE) studies on the samples of effluent were conducted by the Environmental group at the Pulp and Paper Research Institute of Canada.[8] The studies were aimed at ascertaining which effluent was the cause of the toxicity episodes. Conclusions from the TIE were that the toxicity was persistent and was removed by filtration through 1.2 μm filters, autoclaving, and solvent extraction, but it was not removed by
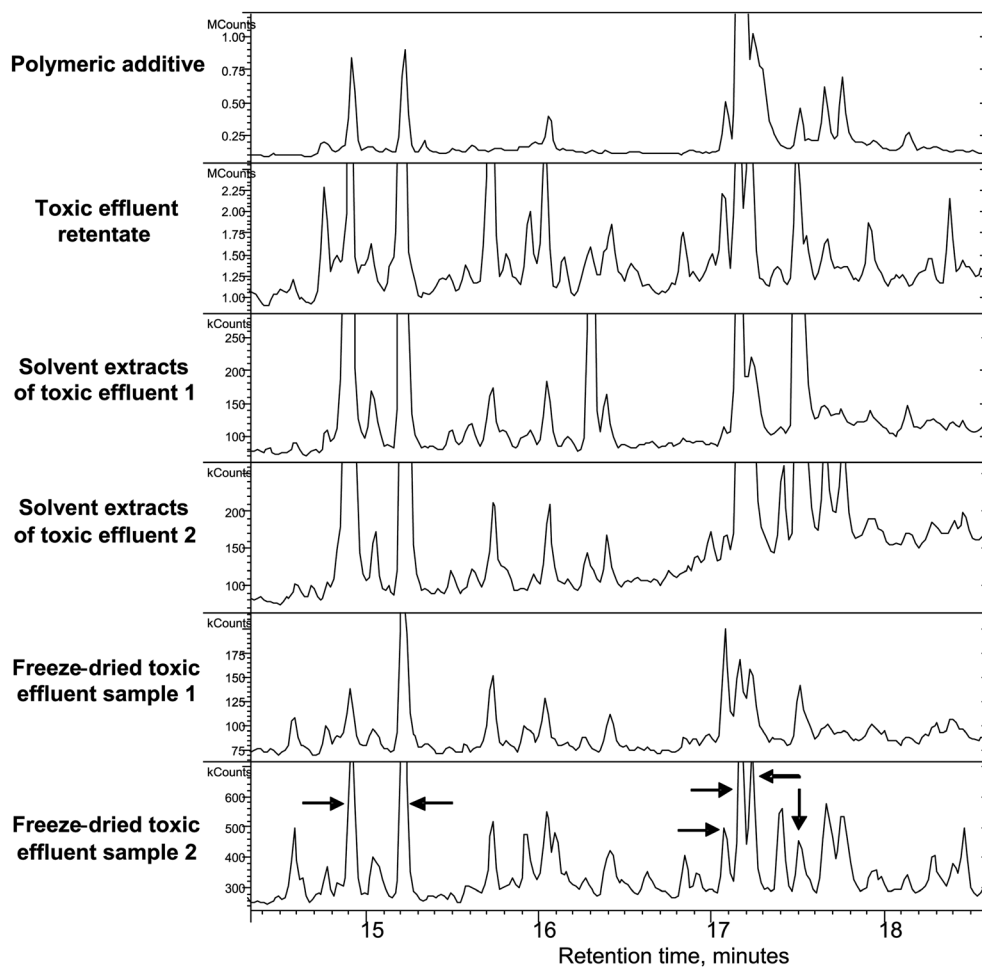

Fig. 1 Pyrogram fingerprints of polymeric additive, solvent extracts of toxic effluents, and freeze-dried solids of toxic effluents. The pyrolysis products that were common in all the samples eluted at 14.91, 15.23, 17.08, 17.17, 17.26, and 17.51 minutes (indicated by arrows).
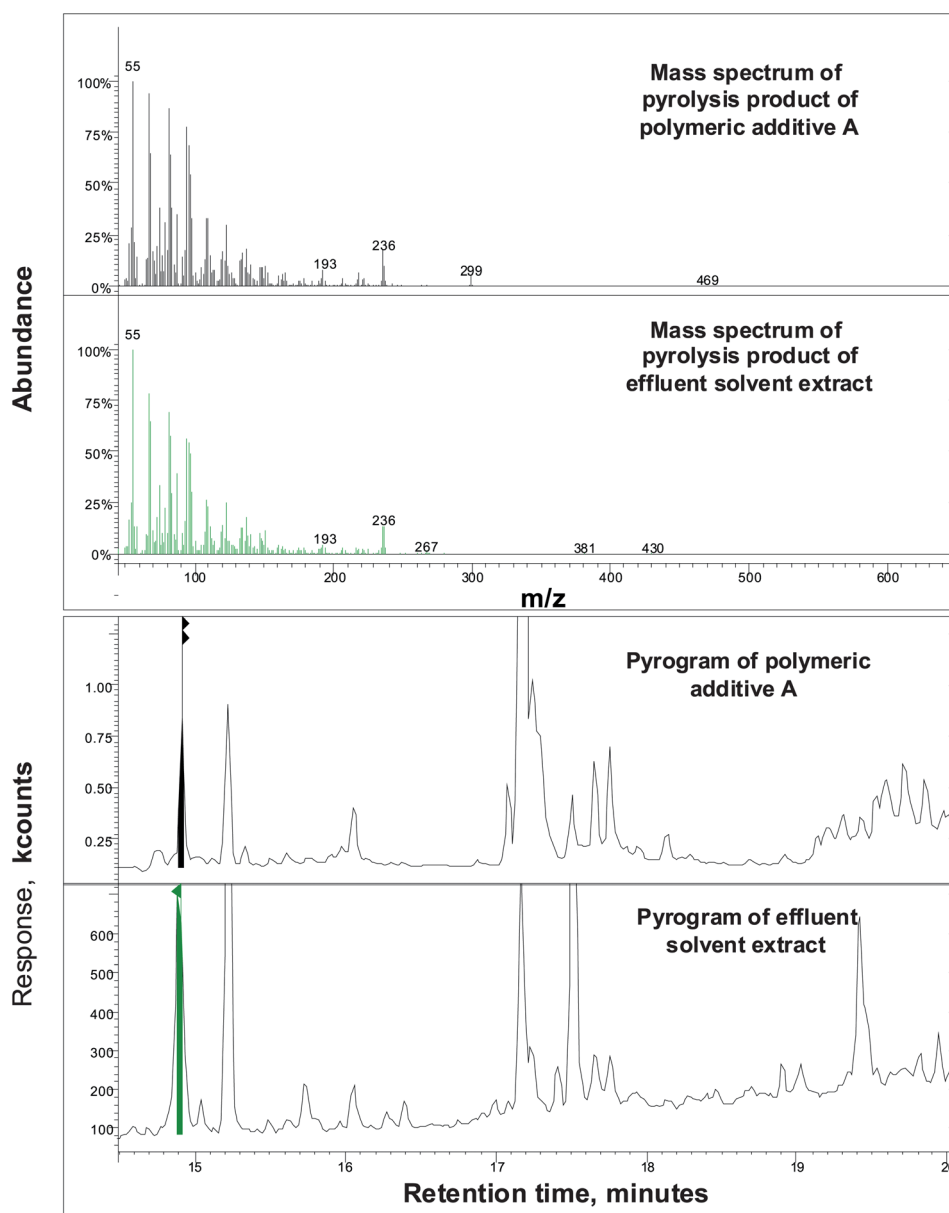
Fig. 2 Fingerprinting of and confirmation for the presence of toxicant material in the solvent-extracted matter and in the polymeric additive using the pyrolysate eluting at 14.1 minutes.
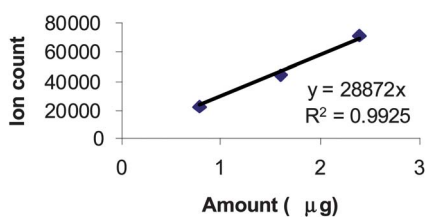

Fig. 3 Calibration curve using the pyrolysis product eluting at 14.91 min.

Fig. 4 Concentration of the toxicant in different sampling points as a function of time.

boiling. An interesting aspect of the studies was that the effluents were toxic to *Daphnia magna* but not to rainbow trout. Also, the toxic material removed by filtration exhibited its toxicity when it was reconstituted in the filtered effluent. The *Daphnia magna* organisms were "coated" with some type of
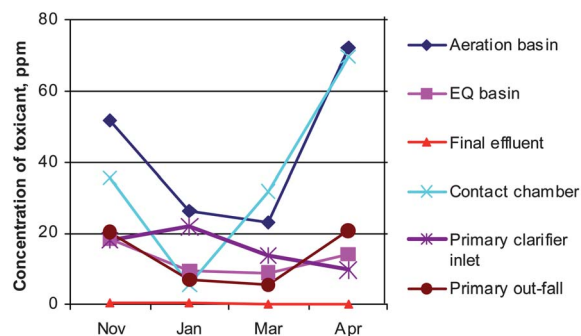
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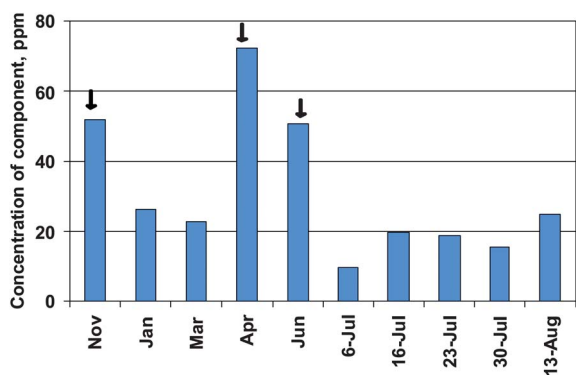*J. Environ. Monit.*, 2012, **xx**, 1–11 | 3

Fig. 5 Monitoring of aeration basin effluent samples: toxicity episodes (marked by arrows) correspond to instances where the concentration levels exceeded 50 ppm.

polymeric materials after the tests. The coating had an amorphous core and appeared to "cross-link" with effluent components. In addition, non-bacterial "strands" were formed. The coating of Daphnia is well known and is commonly used in testing protocols to identify fatal effects on Daphnia when testing the toxicity of low water soluble substances.[8,9] These observations ruled out the usual mill effluent components as the cause of the toxicity. From this it was surmised that the toxicant was an organic chemical, with surfactant properties, that favoured Daphnia due to its chitin exoskeleton structure that may have different surface active properties than the structure of rainbow trout. A polymeric additive was strongly suspected to be the cause of the ongoing toxic episodes. This was bolstered by events at other mills where there had been trials or spills of polymeric additives: the mills were out of compliance during the trials or after the spills and only went
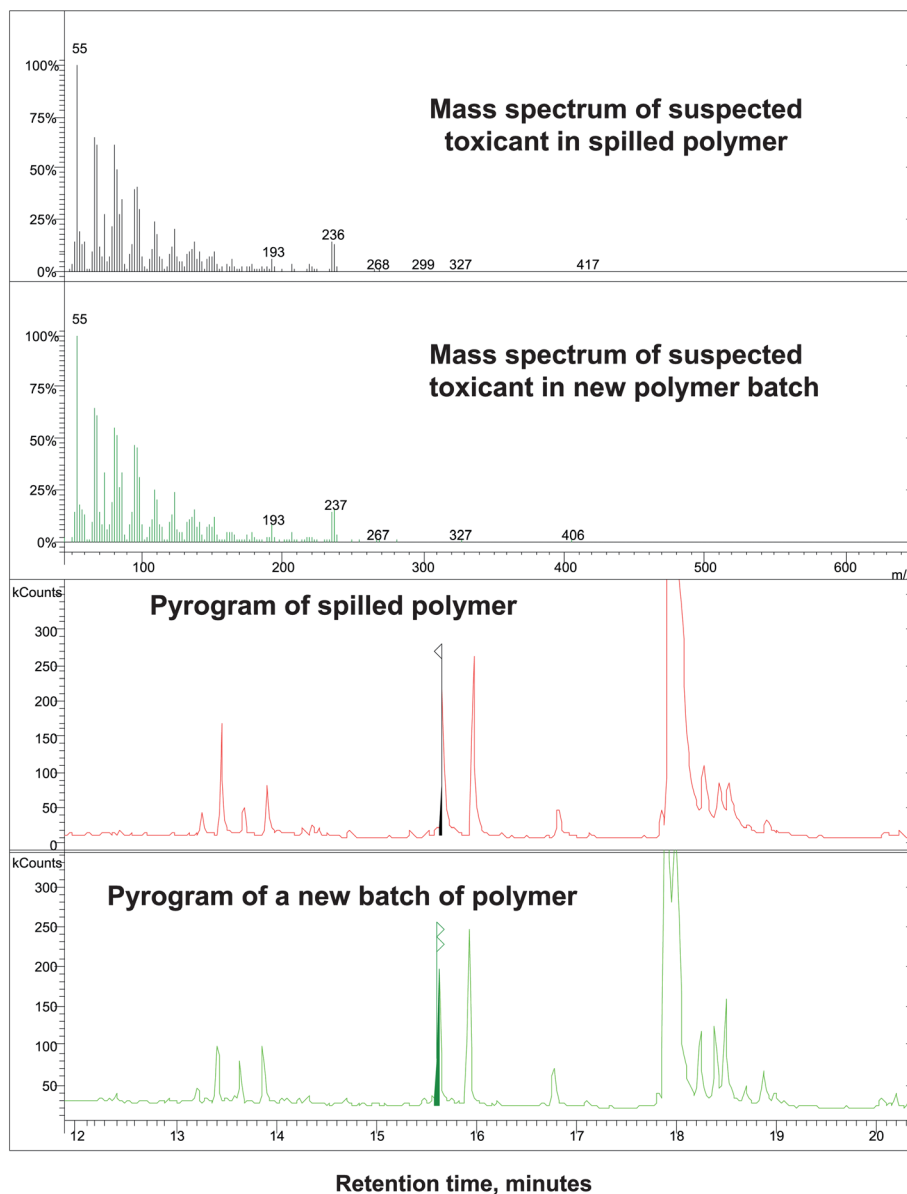

Fig. 6 Comparison of pyrograms and mass spectra of old and new batches of the polymeric additive.

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Table 1 Concentration of toxicant in samples collected from various unit operations of a mill's effluent system

Sample	Concentration of toxicant, ppm (average of 3 replicates)
Paper machine #1, colourless effluent with few suspended solids.	0.01
Paper machine #8, colourless effluent with very little solid at the bottom.	0.20
Dissolved air floatation, colourless effluent with very little solid at the bottom.	0.23
Main sewer, colourless effluent with few suspended solids.	0.33
Primary clarifier inlet, brown effluent with some solids at the bottom.	9.8
Primary clarifier outfall #2, brown effluent with some solids at the bottom.	20.6
Influent feed corn line, brown effluent with some solids at the bottom.	9.4
Equalisation basin, brown effluent with some solids at the bottom.	14.2
Contact chamber, brown effluent with some sludge at the bottom.	69.7
Aeration outfall, colourless effluent with some sludge.	72.3
Return activated sludge, colourless effluent with some sludge.	88.2
Final effluent, colourless effluent with some suspended solids.	0.16
Final effluent dated April 17, colourless effluent with some suspended solids.	0.04
Final effluent (filtered), colourless effluent with some suspended solids.	0.03
Final effluent dated April 17(filtered), colourless effluent with some suspended solids.	0.03
Trash rake residue (dark brown wet solid).	7.6%

back to compliance after returning to the additive used before the trials or corrections of the overdoses.

Experimental

As a polymer was suspect in the toxicity episodes, it was decided to use Py-GC/MS to analyse for it in the effluent. Freeze-dried portions of the mill effluent, before and after filtration, as well as the filtered material were analysed by the technique to ascertain if there were differences in their pyrolysis fingerprint patterns. Additives used at the mill were also analysed to compare their fingerprint profiles with that of the filterable material in the effluents. Later more effluent samples were analysed whenever the toxicity episodes arose.
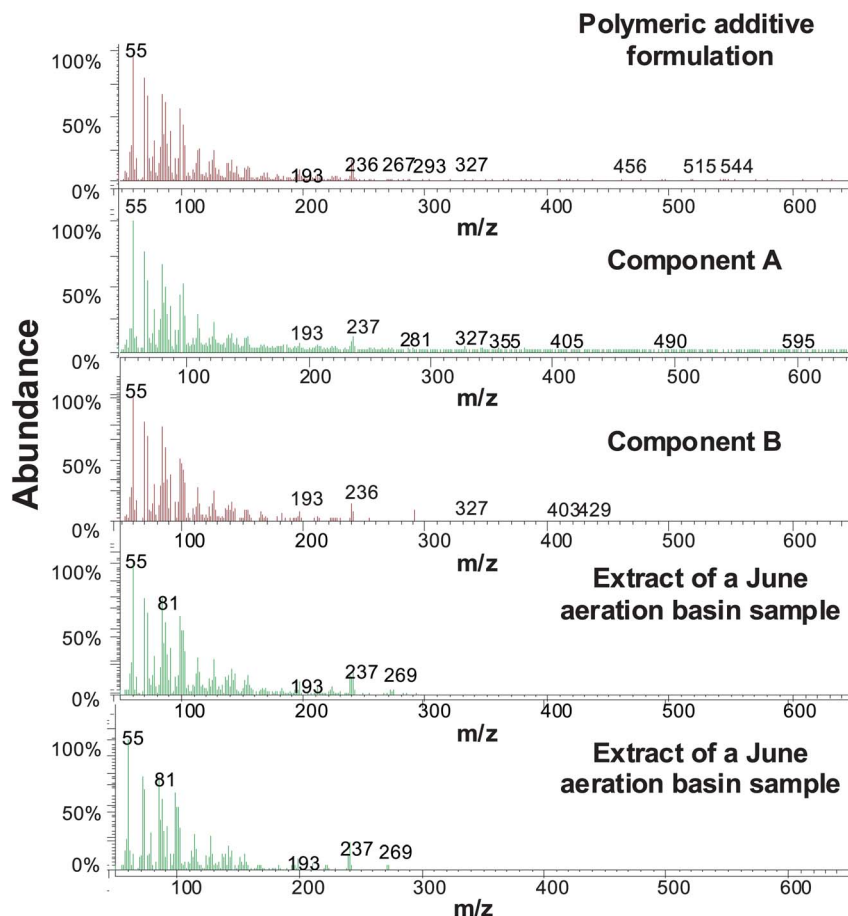
Fig. 7 Comparison of mass spectra of a pyrolysate common in all samples and eluting at 14.91 minutes.

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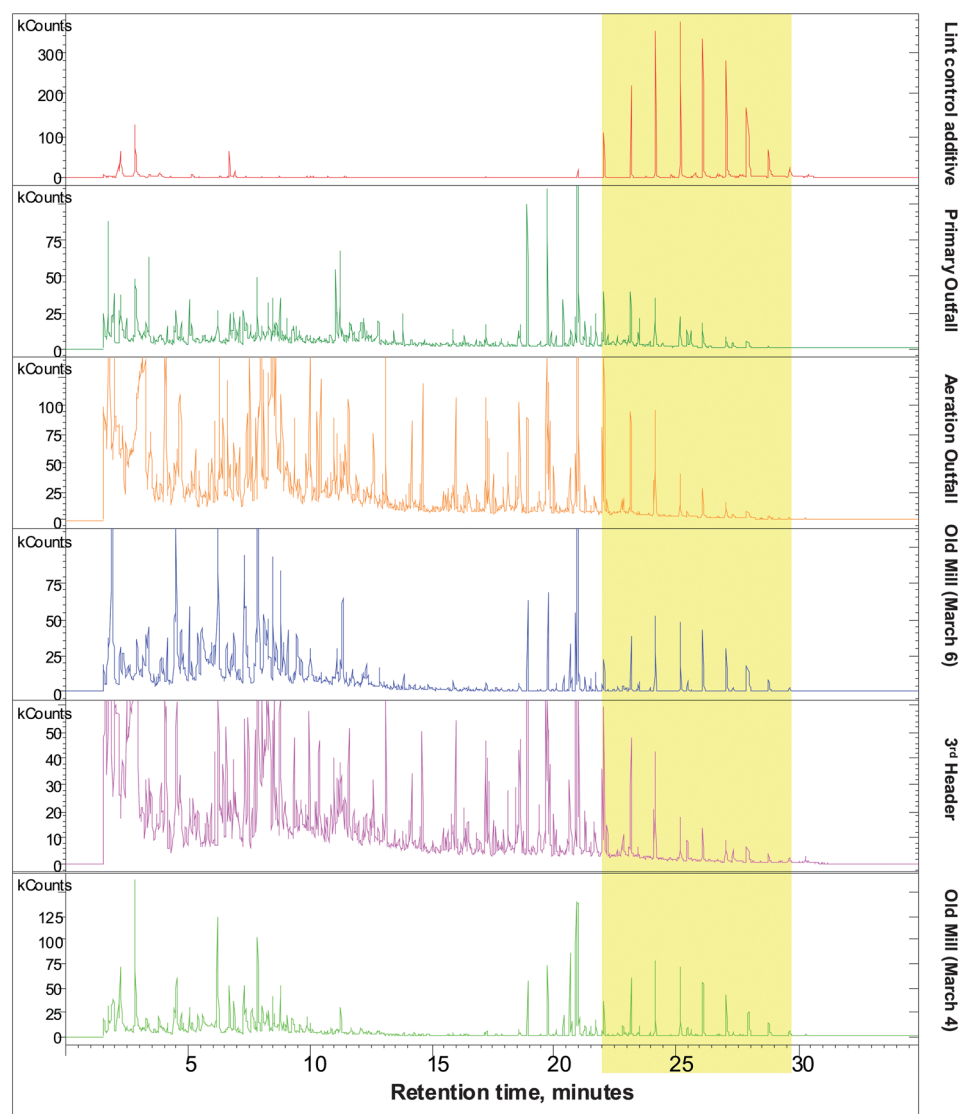
Fig. 8 Comparison of pyrogram fingerprints of lint control additives and suspended solids from effluent samples (the yellow shaded area highlights the pyrolysis patterns of the pyrolysis products common to all the samples analyzed).

Our past experience in studying toxicity of pulp and paper mill effluents has shown that the toxicity can be removed from effluents by extraction with methyl-tertiary butyl ether (MTBE). Thus the toxicant in the effluents was collected by liquid–liquid extraction of 250 mL volumes with 2 consecutive equal volumes of MTBE. Extraction with equal volumes avoided the formation of emulsions. The MTBE extracts were then evaporated down to 100 μL and 5 μL were then analysed by Py-GC/MS.

Analysis of mill additives

Portions were freeze-dried and known amounts (typically 50 μg) were analysed, in triplicate, by Py-GC/MS.

Py-GC/MS

The Py-GC/MS conditions were as follows:

The pyrolyser used was a micro-furnace pyrolyzer with an autosampler (PY-2020iD, Frontier Lab, Japan) attached to a capillary column. Its oven temperature was maintained at 275 °C

and the pyrolysis temperature applied was 650 °C for 20 s. Known amounts of ~3 to 10 μg of samples were loaded into quartz tubes for pyrolysis.

The GC used was a Varian 3900 and the conditions were: injection temperature: 300 °C; injection method: 1 : 25 split; column: DB5-HT: 30 m × 0.25 mm i.d. × 0.10 μm film thickness; flow rate: 1.6 mL min^{-1}; temperature programming: 50 °C for 2 min, 8 °C min^{-1} to 310 °C, hold for 0.5 min.

The mass spectrometer used was a Varian Saturn 2100T ion trap that was used with the following conditions: an interface temperature of 300 °C; an ion trap temperature of 250 °C; electron ionization; and a scan range of m/z 50–650.

Results

Py-GC/MS

At first glance, the pyrogram fingerprint of the solvent extracts of the toxic effluent does not seem to match with that of the spilled polymeric material (see Fig. 1). However, detailed
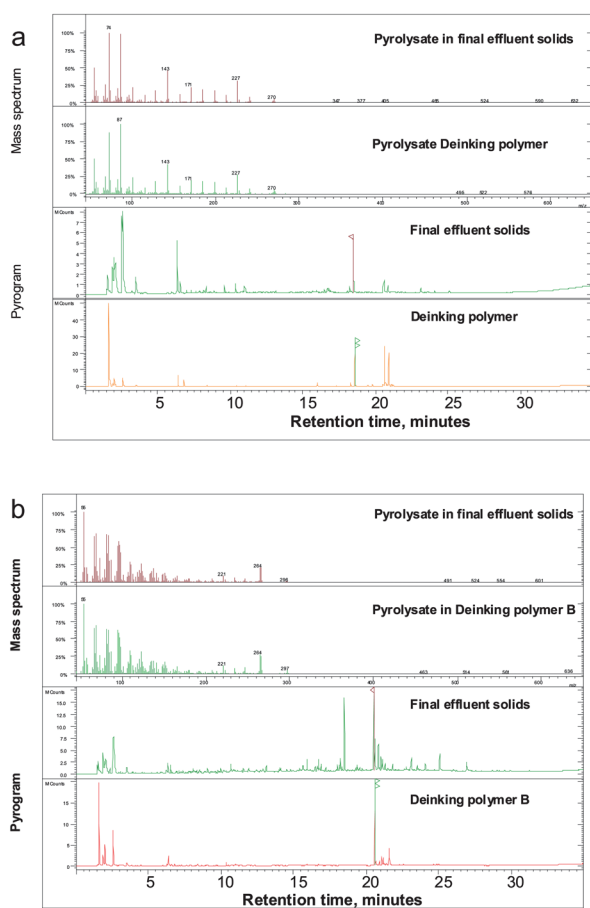
Fig. 9 (a) Fingerprint pyrograms and mass spectra of final effluent solids and a deinking polymer A. (b) Fingerprint pyrograms and mass spectra of final effluent solids and a deinking polymer B.

examination of individual peaks in the pyrograms indicated that there were pyrolysis products that were common in all the samples: they eluted at 14.91, 15.23, 17.08, 17.17, 17.26, and 17.51 minutes. This was confirmed by excellent matches of their mass spectra as illustrated in Fig. 2 for the pyrolysate that eluted at 14.91 min. These pyrolysis products were consistently absent in the extracts of effluents that did not exhibit toxicity to *Daphnia magna*.

The pyrolysate at 14.91 minutes was arbitrarily selected and used for quantification of the toxicant in effluent samples: the calibration was linear as shown in Fig. 3.

Data for quantification of the toxicant in effluent samples collected from various locations over 6 months are shown in Fig. 4. The data show that the amount of toxicant of interest decreased from November to March and then increased in April. It is worth noting that the mill experienced toxicity episodes only with the November and April samples, but not with the January and March samples. The data also showed that samples from the aeration basin exhibited the most toxicity. Consequently, it was decided to monitor this more closely by periodic monitoring of levels of the compound at this sampling point over a long time to ascertain if this could be linked to the toxicity episodes. Data for these analyses are shown in Fig. 5. They seem to indicate that toxicity episodes were experienced only when the concentration of the toxicant exceeded 50 ppm at the aeration basin sampling point.

Samples corresponding to the highest toxicity were studied further and the results are shown in Table 1.

The concentration was particularly high in the trash rake residue at 7.6% in contrast to less than 90 ppm levels in the other samples. The data suggest that the clean-up of the mill equipment caused dislodgement of accumulated matter in the mill unit operations that was rich in toxicant. It is well known that polymeric compounds adsorb very strongly onto surfaces of equipment and accumulated dirt build-ups. Indeed, the high level of toxicant in the trash rake sample at 7.6% supports this hypothesis. The accumulation of toxicant-laden matter in the mill equipment helps to explain the persistent toxicity episodes at the mill: the accumulated matter dislodged periodically and induced effluent toxicity.

A few weeks later the toxicity episodes abated and the mill did not experience any more episodes for quite a long time. From this observation, we surmise that the unscheduled clean-up removed the masses of toxicant-laden matter that were the cause of the toxicity.

Further work to identify the component responsible for the toxicity

As shown in the preceding section, only some of the pyrolysis products of the polymeric additive matched with the fingerprint pattern of the toxicant in the filterable or solvent extracted matter of toxic effluents. This therefore seemed to indicate that some component(s) present in the polymeric formulation contributed to these pyrolysates and thus was the source/cause of the toxicity and not the polymer *per se*. Discussions with the supplier of the chemical revealed that the polymeric additive had been in use in the industry for a long time with no reports of toxicity problems to aquatic biota. However, no data were available to back this up for the formulation used at the mill. A sample of the additive from a fresh batch was then analysed and compared with the spilled batch. The results were identical for both batches and showed the presence of the suspected toxicant as can be seen in Fig. 6. To better understand the cause/source of the toxicity, individual components used in making up the formulation were analysed individually by Py-GC/MS to compare their fingerprints with those of the toxicant in the mill effluents. The results showed that the fingerprint of the pure polymeric additive did not match with the fingerprints of the toxicant in the extracts. However, two of the components used in making up the formulation exhibited fingerprints that matched with that of the suspected toxicant as can be seen in Fig. 7. Thus it appears that the polymeric compound *per se* is not the toxicant, but the formulation contains components that exhibit toxicity to aquatic biota. Since these components were surfactant type compounds their toxicity is not surprising as surfactants are known to exhibit toxicity to aquatic biota.[10,11] Later, fingerprint analyses at other mills that experienced toxicity episodes to *Daphnia magna* also showed this pattern. These episodes occurred when there was overdosing or spills of polymeric additives; the effluents were compliant when the mills returned to normal operating conditions with the additives.
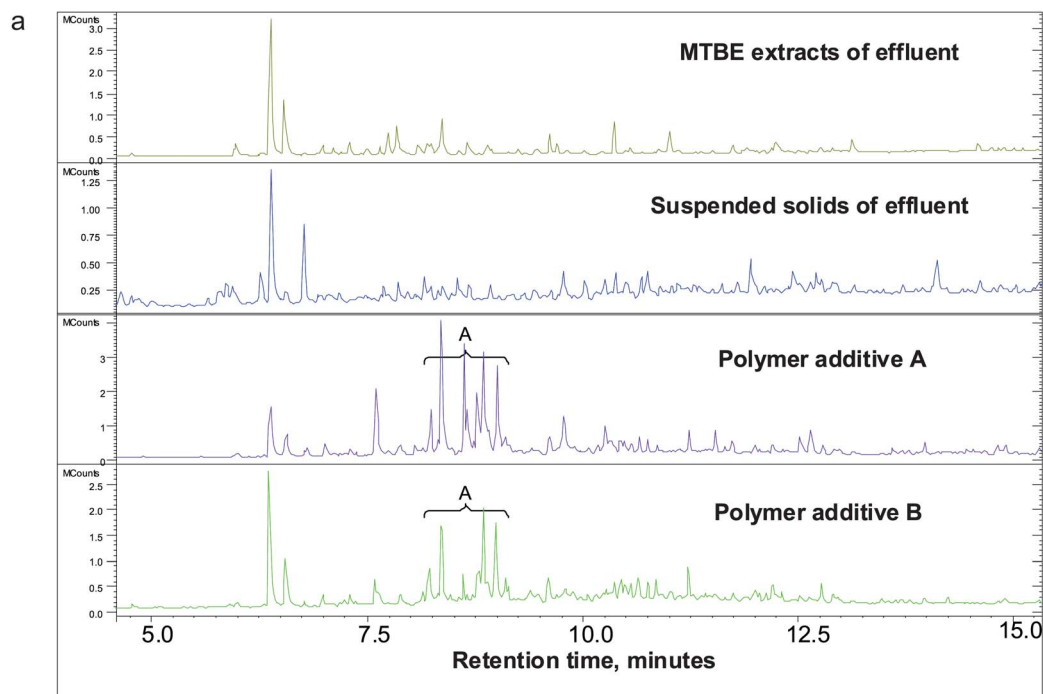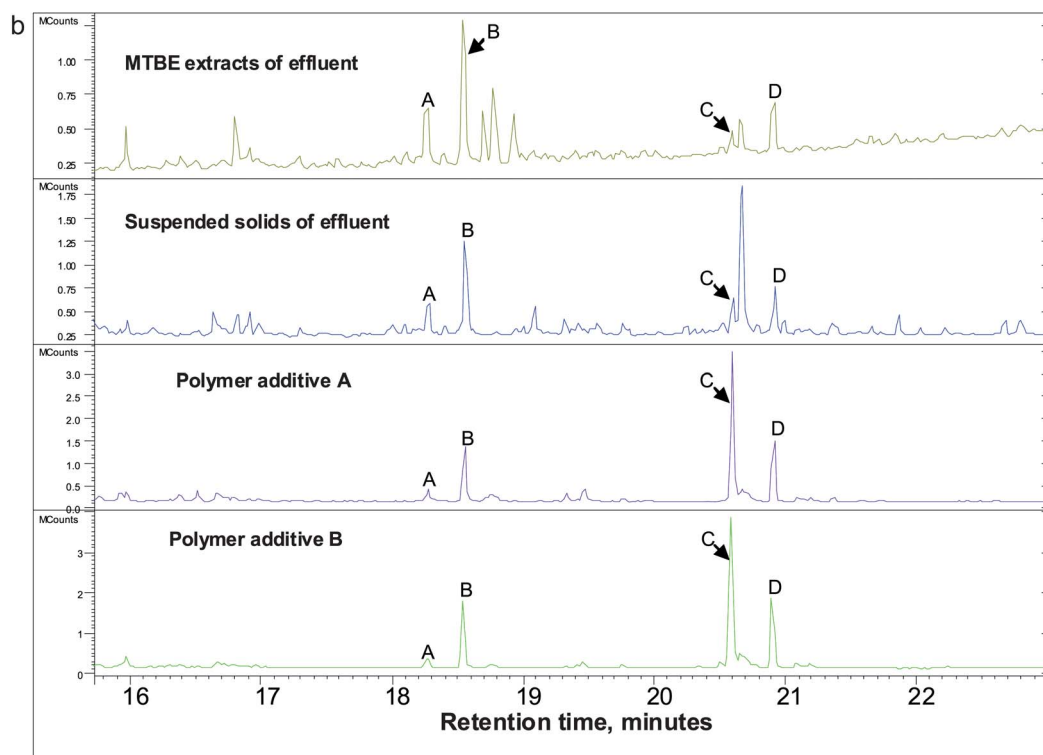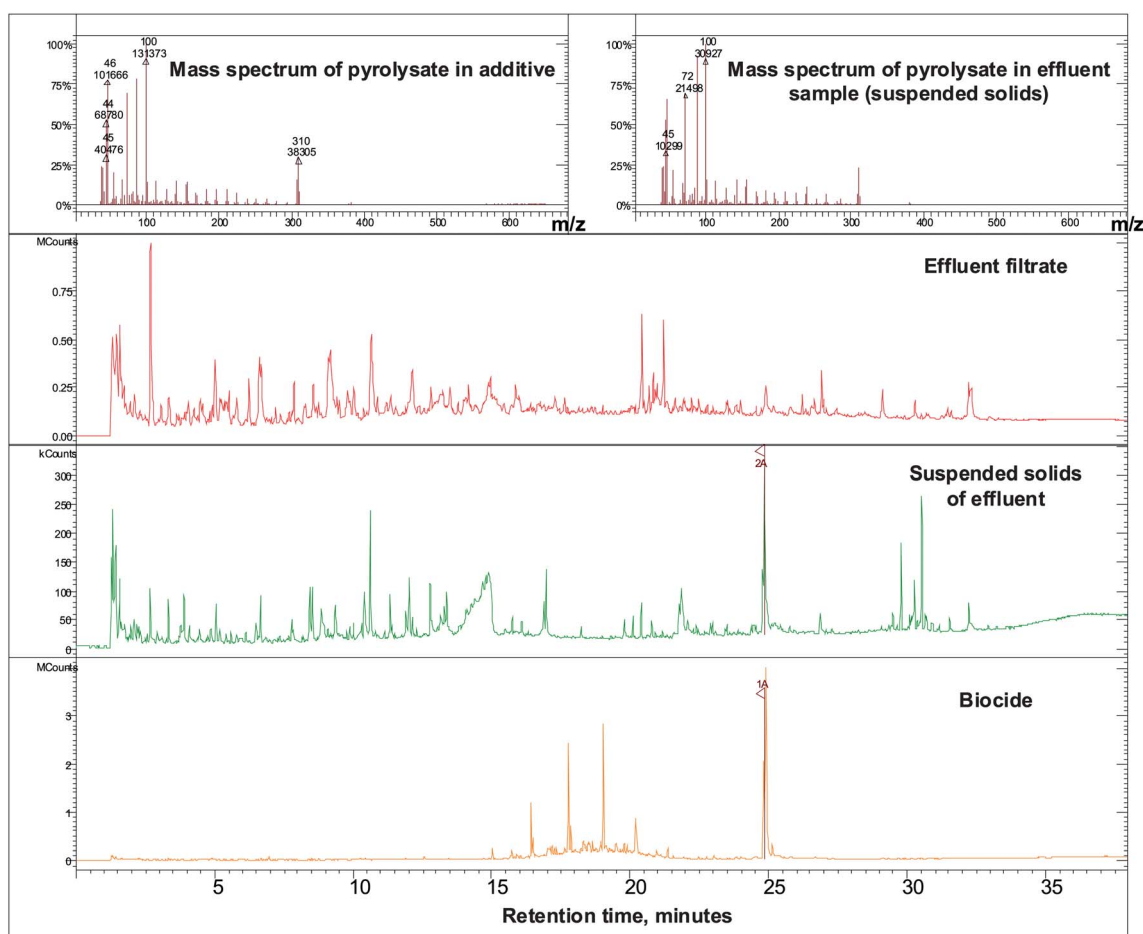
Fig. 10 (a) Fingerprint pyrograms of effluent samples and process additives (the first half of the pyrograms is illustrated). (b) Fingerprint pyrograms of effluent samples and process additives (the second half of the pyrograms is illustrated).

Fig. 11 Fingerprint of a process additive in suspended solids of an effluent sample.

Fingerprinting other additives that caused toxicity to aquatic biota

Py-GC/MS is now used routinely in our laboratory to trouble-shoot toxicity episodes of mill effluents suspected to be caused by additives used at the mills. A few examples are illustrated below.

Example 1: lint control additive in effluent

Effluent samples were analysed to ascertain if any of the several process additives used at the mill were present in the effluents. The samples were filtered through 1.2 μm glass filter papers to remove suspended solids. The samples were also processed by liquid–liquid extraction with MTBE. Analysis by Py-GC/MS showed that one additive, trialed at the mill for use as a lint control agent, was present in the suspended solid portion of all the samples (except the final effluent) and the solvent extracts. This is evident from the pyrograms shown in Fig. 8. Thus it was surmised that the lint control polymer was the cause of the toxicity.

Example 2: deinking polymers in effluent

A mill experienced process upsets in the form of floating foam/sludge on the aeration basin unit. Several samples from various units were collected and analysed for comparison with polymer additives used in the mill. The results showed that surfactants used in the deinking plant were present in the floating sludge samples as illustrated in Fig. 9a and b. Hence it was deduced that carryover of the chemicals from the deinking plant was the cause of the process upsets.

Example 3: coagulant and flocculant additives in effluent

An effluent sample was analysed to ascertain if additives used on paper machines at a recycled paper newsprint mill were responsible for its toxicity. The pyrogram fingerprints of the sample are shown in Fig. 10a. It is evident that the major pyrolysis fragments of the polymers (labeled A) are absent in the solids and MTBE extracts of the effluent. However, the same major components in both the solvent extracts and suspended solids of the effluent sample are also present in the polymer formulations. This can be seen quite clearly in Fig. 10b. The products were fatty acids, products from degradation of the alkyl polyoxyethylene glycol surfactants that were present in the polymer formulations. Thus it appears that the toxicity of the effluents was due to components in the polymer formulations used at the mill.

Example 4: biocides in effluent

Toxicity of a mill effluent was assessed by monitoring for the presence of additives. The effluent sample was analysed to ascertain if the compounds that induced the most toxicity to

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Daphnia at very low levels were present. Comparison analysis of the filtered effluent and its suspended solids with the additives showed that the pyrogram of the suspended solids generated a pyrolysate that was present in three of the additives, namely biocides, which were used at the mill. This pyrolysate was not present in the pyrogram of the filtered effluent as can be seen in Fig. 11. The results therefore indicated that the biocides, or compounds in their formulations, adsorbed onto the suspended solids were the cause of the toxicity of the effluent to *Daphnia magna*.

Conclusions

Py-GC/MS is a powerful analytical technique that can be used to fingerprint additives that cause upsets in operations of mill effluent treatment systems. The presence of the additives is confirmed by fingerprint pyrograms of the additives (or components in the formulations of the additives) in conjunction with mass spectrometry. In this case, the culprit was polyoxy-ethylene glycol surfactants. There is no information in the literature on the toxicity of the polymeric formulation supplied to pulp and paper mills. This report sheds light on this and demonstrates that disposal of excessive amounts of the additive into mill effluents can result in mills failing regulations on toxicity of effluents destined for discharge into receiving waters.

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