Final report

Fate of toxins in hemlock and yew during composting

Measurement of the degradation of plant toxins (coniines in hemlock and taxoids in yew) during a PAS100-compliant commercial green waste composting process.

Project code: OAV031-003 **Date:** August 2010
Research date: July 2009 – January 2010

WRAP helps individuals, businesses and local authorities to reduce waste and recycle more, making better use of resources and helping to tackle climate change.

Written by: David Michie (SAC), Audrey Litterick (Earthcare Technical Ltd.), and Colin Crews (FERA)

Front cover photography: Setting up experimental sample bags in a compost windrow

Executive summary

There is an increasing interest in the use of bulky organic fertilisers, including composts in agriculture. Composts can substitute partly for crop synthetic fertiliser requirement, and also act as soil conditioners. Their increased popularity in agriculture is due to increased availability, the documented benefits that they can bring to soils and crops, and recent increases in inorganic fertiliser prices. Recent risk assessments have highlighted a lack of understanding of the fate of plant toxins in hemlock (*Conium maculatum*, coniines) and yew (*Taxus baccata,* taxoids) during green waste composting processes. This has caused some concern within the livestock sector in Scotland as to the safety of composted green wastes applied to land that may subsequently be used for grazing livestock.

Project aims

To determine whether, and to what extent, coniines in hemlock plant material and taxoids in yew plant material degrade over the duration of a PAS100-compliant commercial green waste composting process. Samples of hemlock and yew material were held in net bags which were placed in a composting windrow. Windrow temperatures were monitored throughout the duration of the composting period, and test samples were retrieved at intervals governed by the windrow turning regime. Test samples of hemlock were analysed for concentrations the coniines 'coniine' and 'coniceine', while all yew samples were tested for concentrations the taxoids 'taxine A' and 'taxine B'. Analyses were performed by liquid chromatography time-of-flight mass spectrometry (LC-ToF-MS).

The main findings can be summarised as follows

- Taxoids (taxines A and B) present in yew leaves and branches were degraded to below the limits of detection (LOD) after 65 days of the PAS 100 composting process.
  - The degradation of taxoids in yew plant tissues was accelerated by the composting process.
- Concentrations of coniine present in hemlock tissue decreased by 64 % from the time of the first measurement (turning 2) until the end of the 20 week composting process, and by 92 % over the same period when left exposed to outside weather conditions.
- Concentrations of coniceine present in hemlock tissue decreased to below the limit of detection after 5 weeks both during the 20 week composting process and when left exposed to outside weather conditions for the same time period.
  - The degradation of coniine in hemlock plant tissues was not accelerated by the composting process.
- Given the likelihood of partial degradation prior to composting, degradation during the composting process and the likely dilution of yew and hemlock in typical PAS100 compost feedstocks, the toxins present in yew and hemlock are expected to pose a negligible risk to livestock grazing on compost-treated fields.

Contents

1.0 Introduction and objectives **3**
1.1 Objectives 4
2.0 Materials and Methods **4**
2.1 Location and duration of the trial 4
2.2 Plant material used 4
2.3 Compost characteristics 4
2.4 Preparation of samples 4
2.5 Trial details 5
2.6 The composting process 5
2.7 Measurements and testing methods 5
2.7.1 Toxin concentrations 5
2.7.2 Internal windrow temperature 6
2.7.3 Windrow moisture content 6
2.7.4 Statistical analysis of toxin concentrations 6
3.0 Results **6**
3.1 Coniine and coniceine concentrations 6
3.2 Taxine concentrations 8
3.3 Internal windrow temperature 11
3.4 Windrow moisture content 11
4.0 Discussion **13**
5.0 Implications for commercial growers/farmers **15**
6.0 Recommendations for future work **15**
7.0 Acknowledgements **15**
References **16**

1.0 Introduction and objective

Around 4.5 million tonnes of source-segregated organic waste were composted in the UK in 2007/08 (AFOR, 2009). Most of the feedstock currently composted is composed largely of green (or botanical) wastes from municipal collections, from local authority parks and gardens, and from commercial landscapers.

The safety and quality of composts is of prime importance, especially when they are to be used as soil improvers on land used to grow crops for human consumption or grass for livestock grazing; or as components of growing media that will be handled during use. Almost half (47%) of all the compost produced from source-segregated organic wastes in the UK (i.e. 1.3 million tonnes) is used in agriculture (this includes livestock production, arable crops and field horticultural crops, but not growing media or container plant production), and around 71% of compost producers supplied compost to this market sector in 2007/08 (AFOR, 2009).

More than half of source-segregated organic wastes collected for composting in the UK are processed under the PAS100 rules, which require the adoption of a HACCP (Hazards Analysis and Critical Control Points) framework for compost producers to understand and codify quality control processes specific to each composting site in a set of standard operating procedures. This details the quality management system for the entire composting process, and PAS also prescribes minimum quality criteria for composts produced within the framework of the specification. Concerns have recently been raised about the potential hazards which might be associated with compost, and which are not currently addressed by process validation or minimum quality criteria within the PAS100 specification. One such concern was the possibility of toxins, naturally synthesized within some garden and wild plant species, persisting throughout the composting process and causing harm to persons handling the compost, or to livestock grazing on compost-treated land. Several poisonous plant species, for example rhododendron (*Rhododendron* species), and foxglove (*Digitalis* species) are commonly grown in UK gardens, and are therefore likely to be present in compost feedstocks.

During a recent risk assessment conducted by the Macaulay Research Institute (Macaulay, 2009) three plant species were raised as potential concerns due to a lack of information on the degradation of toxins present within them. These were rhododendron (*Rhododendron* spp.), yew (*Taxus baccata*) and hemlock (*Conium maculatum*). A project to examine the fate of toxic compounds in rhododendron was completed in 2009, demonstrating that grayanotoxin I and grayanotoxin III completely degraded within 70 days, and rhododendron did not therefore pose a risk to human or animal health if fully composted within a PAS100-compliant process (WRAP, 2009). Vegetative material of hemlock was not available at the time that the rhododendron research was undertaken, and this project seeks to understand the fate of toxic compounds in both hemlock and yew. Yew is a native evergreen conifer which is frequently planted in parks and domestic gardens. It has been seen to occur in small amounts in feedstock mixes awaiting shredding at composting sites (A Litterick, personal experience). Hemlock is a weed of moist soils and damp places such as streamsides, field edges and waste ground, and is extremely unlikely to occur in parks and gardens. As with other species (such as ragwort, *Senecio jacobaea*) that occur on less-managed land, there is no incentive for such plant materials to be sent for composting after cutting, since this incurs a fee – it is far more likely that cut material will be left to decay in situ.

The principal toxins in hemlock are known as coniines, and include coniine and coniceine. Concentrations of these toxins present in plants have been found to vary both with the site that the plants were taken from, and with the plant part tested (Lopez *et al*, 1998). Coniceine has been shown to readily convert to coniine, probably due to environmental conditions (Leete and Adityachaudhury, 1967). The principal toxins in yew are known as taxoids, and include taxines A and B. Taxine B is the major alkaloid responsible for the toxicity of the European yew (Wilson *et al*, 2001).

The authors have not found any refereed reports on the degradation of toxins in either yew or hemlock during a composting process. This project examines the effect of a typical outdoor windrow composting process on the concentrations of plant toxins present in *Taxus baccata* and *Conium maculatum* plant material. The implications of the results are discussed with particular reference to the safety of composts for grazing livestock.

1.1 Objective

The objective of this project was to examine the rate of degradation of coniine and coniceine (in hemlock samples) and taxines A and B (in yew samples) composted in a working windrow on a PAS100 accredited composting site. Control samples of both hemlock and yew were held at outdoor ambient conditions (i.e. they were exposed to rain, wind and ambient temperatures (and were not composted). The temperature and moisture content of the composting windrow were recorded as accurately as possible throughout the duration of the trial in order to allow an assessment of how these contributed to degradation.

2.0 Materials and Methods

2.1 Location and duration of the trial

This trial was conducted at Fife council's Lochhead landfill site and recycling centre (Drumtuthill Road, Wellwood, Dunfermline, Fife, KY12 0RX), a commercial, PAS100-accredited composting facility that composts green waste in outdoor windrows. The trial took place over a 20 week composting period.

2.2 Plant material used

Fresh yew hedge trimmings were taken from mature growing plants at Pitmedden Gardens, Aberdeenshire on the 20th July 2009. This material was then measured out as 35 g subsamples, and placed in prepared nylon net bags ('subsample bags', see Section 2.4) along with 35 g shredded mixed green waste (that had been tested and found to contain none of the plant toxins under investigation in this project) on the 21st July 2009.

Fresh hemlock plants were taken from a site in Oxfordshire, England. Despite several hours of searching in likely places, guided by the vice-county plant recorder in Aberdeenshire, no hemlock was found in Aberdeenshire or Angus. This hemlock material comprised stem, leaf and flower material, all of which was lightly shredded with secateurs, measured out as 35 g subsamples, and placed in prepared subsample bags along with 35 g shredded mixed green waste (that had been tested and found to contain none of the plant toxins under investigation in this project) on the 22nd July 2009.

2.3 Compost characteristics

The green waste which made up the host windrow was brought onto the site between the 11th and the 24th July 2009. The majority of this waste was from municipal (household) collections, and the remainder was brought to the site by commercial landscapers. The feedstock load which made up the host batch used for the trial was examined in detail on the compost pad, and no evidence of either hemlock or yew was found. Shredding took place on the 24th July, and the windrow was formed on the 30th July 2009. The compost was turned eight times during the 12 week managed composting process (Table 1). The compost was matured with a further two turns during the subsequent 8 week maturation phase. Screening took place following maturation.

2.4 Preparation of samples

Subsample bags were prepared by cutting 20 cm x 20 cm strips of nylon mesh (mesh size 1.5 mm) and sewing them together on three sides with non-biodegradable polyester thread using a 0.5 mm seam. After they had been filled with plant sample materials they were sewn shut (again with polyester thread using a 0.5 mm seam) and a nylon fluorescent tag was attached. Each fluorescent tag was given the same number as the main bag into which it was placed (see below), using indelible pen and notches cut into the tag. Fifty-five subsample bags of yew and fifty-five of hemlock were prepared in this way.

Table 1 Bags retrieved at each turning from the composting windrow at Lochhead landfill site

Turning number	Number of days composting	Bags from which subsample bags were taken	
		Hemlock	Yew
1	5	none	None
2	12	5, 7, 8, 9, 11	5, 7, 8, 9, 11
3	27	3, 5, 6, 9, 11	3, 6, 8, 9, 11
4	40	none	None
5	53	1, 3, 4, 6, 11	1, 3, 6, 7, 11
6	65	3, 6, 8, 10, 11	3, 6, 8, 10, 11
7	75	1, 2, 4, 10, 11	1, 2, 4, 10, 11
8	86	5, 6, 8, 10, 11	5, 6, 8, 10, 11
9	102	1, 3, 4, 10	1, 3, 4, 10
10	118	none	None

Eleven large polypropylene net bags ('main bags') measuring 45 cm x 60 cm with drawstring tops (mesh size 12 x 6 x 3 mm), were then each filled with five hemlock and five yew subsample bags, and attached to a nylon fluorescent tag (110 x 380 mm) with a 50 cm length of fluorescent yellow 3 mm polypropylene rope in order to aid identification. Each tag had a number written on it using permanent marker as well as a series of notches cut out of the tag that corresponded with the bag's number for identification. A 12 mm diameter polypropylene rope measuring around 10 m in length was tied to the neck of each main bag.

2.5 Trial details

Ten of the main bags were distributed evenly throughout the host windrow (batch reference number 012/2009) at Lochhead composting site on the 30th July 2009 at the time of the first turning. The 10 m lengths of rope were left trailing out of the windrow in order to aid location of the bags at subsequent turnings. The control samples in the eleventh main bag were suspended from a wooden frame by the edge of the compost pad, where they were allowed to remain at ambient meteorological conditions for the full 20 weeks of the composting process.

Each of the eleven main bags were retrieved from the windrow during each turning, checked for damage and repaired if necessary, then replaced at regular intervals and at various heights within the windrow. The aim was to place samples throughout the cross-section of the windrow in order to most closely approximate what would normally happen with feedstock. A subsample bag of both hemlock and yew were retrieved from four randomly selected main bags in the windrow at seven of the eight windrow turns during the actively managed composting period, and once during the stabilisation period. A subsample bag from the outdoor control main bag was taken at six of these same eight times (Table 1). Only a single subsample bag from the outdoor control main bag was taken at each sampling event to limit expenditure. Subsample bags were tested for plant toxins at the EN 45011 accredited Food and Environment Research Agency laboratories (FERA, Sand Hutton, York, YO41 1LZ).

2.6 The composting process

Fife council mechanically turned the test windrow using a digger excavator eight times within the 12 week actively managed composting period. This 12 week phase consisted of an initial sanitisation phase, followed by a stabilisation phase. In accordance with the standard operating procedure used on the site, sanitisation was considered complete once the windrow had been shown to have an average temperature in excess of target (in this case 65^{o}C) for seven (not necessarily consecutive days). The remainder of the 12 week actively managed composting period was termed the stabilisation phase. The windrow was also turned twice during the following 8 week maturation phase.

2.7 Measurements and testing methods

2.7.1 Toxin concentrations

All hemlock samples were tested by FERA for the coniines 'coniine' and 'coniceine', and all yew samples were tested for the taxoids 'taxine A' and 'taxine B' using liquid chromatography time-of-flight mass spectrometry (LC-ToF-MS; Dass, 2007; McMaster, 2005). This method provides accurate molecular mass information, allowing empirical formulae to be matched against a database without the need for authentic standards (Crews *et al*, 2009). The coniine data was converted to mg/kg of dry sample using a coniine standard to measure the response for a known concentration. The taxoid data consisted of measurements of the chromatographic peak area of the LC-ToF-MS chart and was not converted to mg/kg as there was no taxine standard available.

2.7.2 Internal windrow temperature

One subsample bags in each main bag contained a Tinytag TK-4014-V1 temperature logger. These data loggers were programmed to take a temperature reading every 6 hours over a 20 week period, to allow the determination of temperature trends within the windrow over time. They were designed to allow data to be downloaded directly to computer after use. Fife council also took four temperature readings per working day (during the sanitisation phase of the composting process) and four readings per week (during the stabilisation phase of the composting process) using a temperature probe that was inserted 1.5 m into the windrow. No temperature readings were taken by Fife Council during the maturation phase.

2.7.3 Windrow moisture content

The moisture content of the windrow was estimated and scored by a trained member of the composting site staff using the 'squeeze test' (a standard and accepted practice on UK PAS100 composting sites, Table 2). This test was carried out once a week for each of the weeks throughout the duration of the 12 week actively managed composting period.

Table 2. Moisture assessment index for compost.

Index no.	Moisture behaviour in sample when a handful of compost is grasped and clenched in a gloved hand for ~ 10 seconds, then opened.	Interpretation
1	Water seeps out	Too wet (>65 % moisture)
2	More than one droplet appears	Too wet (>65 % moisture)
3	One droplet appears	OK (40 – 65 % moisture)
4	Compost particles remain packed together and no droplets appear	OK (40 – 65 % moisture)
5	Compost particles fall away from each other	Too dry (<40 % moisture)

The ability of a compost producer/member of site staff to achieve a correct estimation of the compost moisture content using the above test must be annually checked against results obtained in a lab, under the terms of the UK Compost Accreditation Scheme.

2.7.4 Statistical analysis of toxin concentrations

Results from toxin testing were statistically analysed (using T tests) to determine whether there were significant differences between treatments on individual sampling dates). It was not possible to determine whether differences existed between control sample bags placed outside the windrow, due to (statistically) insufficient sample numbers.

3.0 Results

3.1 Coniine and coniceine concentrations

The mean concentrations of coniine measured in the composted and uncomposted *C. maculatum* material both decreased over time. Concentrations of coniine measured in hemlock material which had been placed in the compost windrow averaged 1.32 mg/kg of sample after 5 days composting, whereas those in the control bag measured 8.1 mg/kg (Table 3).

Table 3. Coniine concentrations (mg/kg of sample) in hemlock samples removed from a PAS100 compost windrow over time. (nd= no coniine was detected) Average concentrations with different superscript letters are significantly different from one another ($P \leq 0.01$).

	Number of days (and turns) after the start of composting							
	5 (2)	27 (3)	53 (5)	65 (6)	75 (7)	86 (8)	102 (9)	143 (end)
Bag 1			28.1		17.9		6.45	0.93
Bag 2					7.85			0.70
Bag 3		nd		24.8			1.75	
Bag 4			27.5		10.0		1.90	0.15
Bag 5	3.48	nd				1.50		
Bag 6		nd	37.2	21.1		4.95		
Bag 7	0.30		25.2					
Bag 8	0.50			32.0		5.10		0.12
Bag 9	1.00	nd						
Bag 10				26.5	4.45	4.95	2.65	
Mean conc.	**1.32**[a]	**nd**	**29.5**[c]	**26.1**[c]	**10.1**[b]	**4.13**[a]	**3.19**[a]	**0.48**[a]
Bag 11	8.10		22.2	22.4	4.25	4.90		0.67

No coniine was detected in the composting hemlock samples when they were retrieved after 27 days. It was decided to discard this data as an outlier on the advice of the chemist in the lab (Colin Crews, team leader toxicants and contaminants processing team, FERA, personal communication).

Coniine concentrations increased to an average of around 29 and 26 mg/kg sample after 53 and 65 days of composting respectively. These concentrations were slightly higher than those measured in the control samples tested at the same time. Coniine concentrations in both the composting samples and in the control samples decreased over time thereafter, though concentrations were similar in the composting samples and the control on each sampling date where both were tested.

No coniceine was detected (nd) in either the composted hemlock samples or the control samples 5 or 27 days after the experiment began (Table 4). Coniceine was detected in similar concentrations in the control and composted hemlock samples 53 days after the start of the experiment, but it was not detected thereafter in any samples.

Table 4. Coniceine concentrations (mg/kg of sample) in hemlock samples removed from a PAS100 compost windrow over time. (nd= no coniceine was detected)								
	Number of days (and turns) after the start of composting							
	5 (2)	27 (3)	53 (5)	65 (6)	75 (7)	86 (8)	102 (9)	143 (end)
Bag 1			1.20	nd	nd		nd	nd
Bag 2				nd	nd			nd
Bag 3		nd	0.45				nd	
Bag 4				nd	nd		nd	nd
Bag 5	nd	nd				nd		
Bag 6		nd	0.55			nd		
Bag 7	nd		0.20					
Bag 8	nd					nd		nd
Bag 9	nd	nd						
Bag 10				nd	nd	nd	nd	
Mean conc.	**nd**	**nd**	**0.60**	**nd**	**nd**	**nd**	**nd**	**nd**
Bag 11	nd		0.87	nd	nd	nd		nd

3.2 Taxine concentrations

Concentrations of taxine A measured in samples of yew material which had been placed in the compost windrow averaged 88 999 (chromatographic peak area) after 5 days composting, whereas those in the control bag measured almost 6 000 000 (Table 5). Concentrations of taxine A measured in samples of yew material retrieved from the windrow during the composting period dropped over time until they were no longer detectable in material which had been composted for 65 days. In contrast, concentrations of taxine A in the control sample, which had been kept under ambient environmental conditions was 1 291 801 after 65 days and 571 996 after 143 days. Taxine A had therefore completely degraded by 65 days in the composted yew material, but it was still present in un-composted yew clippings after 143 days.

Concentrations of taxine B measured in samples of yew material which had been placed in the compost windrow averaged 21 387 (chromatographic peak area) after 5 days composting, whereas those in the control bag measured almost 3 271 700 (Table 6). Concentrations of taxine B measured in samples of yew material retrieved from the windrow during the composting period dropped over time until they were no longer detectable in material which had been composted for 65 days. In contrast, concentrations of taxine B in the control sample, which had been kept under ambient environmental conditions was 3 421 814 after 65 days and 1 057 092 after 143 days. Taxine B had therefore completely degraded by 65 days in the composted yew material, but it was still present in un-composted yew clippings which had lain outdoors, outside the compost windrow, after 143 days.

Table 5. Taxine A concentrations (mass spectrometry peak areas) in yew samples removed from a PAS100 compost windrow over time. Average concentrations or concentrations in Bag 11 with different superscript letters are significantly different from one another ($P \leq 0.01$).

	Number of days (and turns) after the start of composting							
	5 (2)	27 (3)	53 (5)	65 (6)	75 (7)	86 (8)	102 (9)	143 (end)
Bag 1			15,562	nd	nd		nd	nd
Bag 2				nd	nd			nd
Bag 3		nd	nd				nd	
Bag 4			nd	nd	nd		nd	nd
Bag 5	9,471					nd		
Bag 6		nd	nd			nd		
Bag 7	168,526							
Bag 8	nd	22,474				nd		nd
Bag 9	nd	nd						
Bag 10				nd	nd	nd	nd	
Mean conc.	**88,999[a]**	**22,474[a]**	**15,562[a]**	**nd**	**nd**	**nd**	**nd**	**Nd**
Bag 11	5,955,489	43,772,465	30,066,525	1,291,801	1,543,118	483,399		571,996

Table 6. Taxine B concentrations (mass spectrometry peak areas) in yew samples removed from a PAS100 compost windrow over time. Average concentrations or concentrations in Bag 11 with different superscript letters are significantly different from one another (P ≤ 0.01).

	Number of days (and turns) after the start of composting							
	5 (2)	27 (3)	53 (5)	65 (6)	75 (7)	86 (8)	102 (9)	143 (end)
Bag 1			998	nd	nd		nd	nd
Bag 2				nd	nd			nd
Bag 3		nd	nd				nd	
Bag 4			490	nd	nd		nd	nd
Bag 5	5,599					nd		
Bag 6		nd	nd			nd		
Bag 7	37,174							
Bag 8	nd	1,702				nd		nd
Bag 9	nd	nd						
Bag 10				nd	nd	nd	nd	
Average conc.	**21,387[a]**	**1,702[b]**	**744[c]**	**nd**	**nd**	**nd**	**nd**	**Nd**
Bag 11	3,271,700	34,325,809	81,342,884	3,421,814	2,761,674	774,693		1,057,092

3.3 Internal windrow temperature

All of the data loggers that had been inserted into the main bags that were placed in the composting windrow malfunctioned to the extent that it was not possible to salvage any temperature data from them (this is discussed in Section 4). The data logger in the control bag, which had been maintained in air ambient temperatures outside the compost windrow functioned as intended. This was extremely regrettable, since it means that the expected temperature data resolution (i.e. that associated with each sample bag) could not be obtained. However, since the site operators were able to provide their own temperature data (at a lower resolution across the windrowed material), it is still possible to determine general trends in the decay of the toxic compounds over time. All of the temperature data presented were obtained from manual probes used by Fife Council (see Section 2.7.2) or from the functioning data logger in control bag (Bag 11) (Figure 2).

Compost temperature within the windrow rose rapidly from air ambient temperatures (around 6°C) to around 70°C in the 2 days following batch formation. The mean windrow temperature (based on four measurements taken at different points along the windrow to 1.5 m depth) remained around 70°C for over 5 weeks after batch formation (excluding the brief drops in temperature associated with turning the windrow). Temperatures remained between 60°C and 78°C for a further 6 weeks, and only dropped below 60°C after 12 weeks of composting (see Figure 2 overleaf).

3.4 Windrow moisture content

The moisture content of the compost windrow was measured as being between 40 – 65 % throughout the duration of the 20 week composting process (Figure 1).

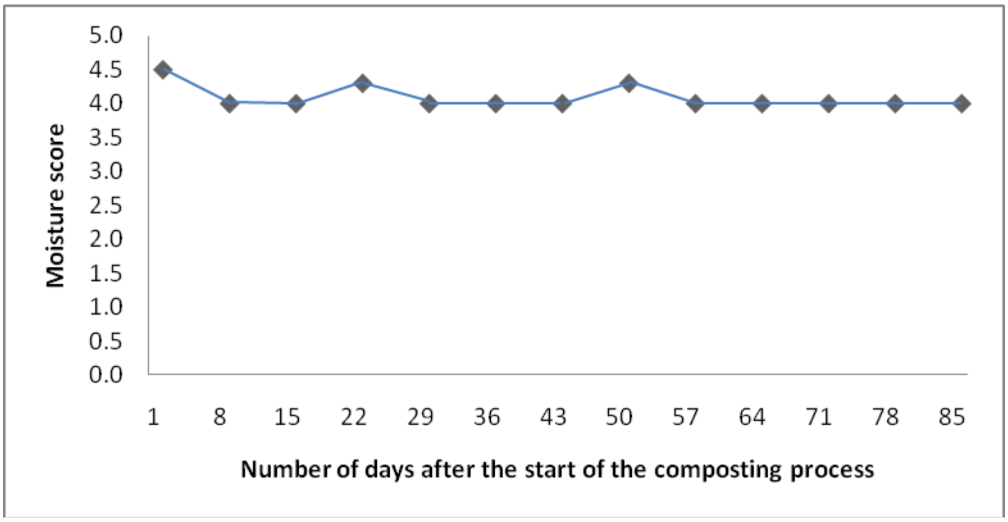

Figure 1. Moisture content of the composting windrow recorded during the composting process. Moisture readings were taken weekly during the 12 week actively managed composting period.

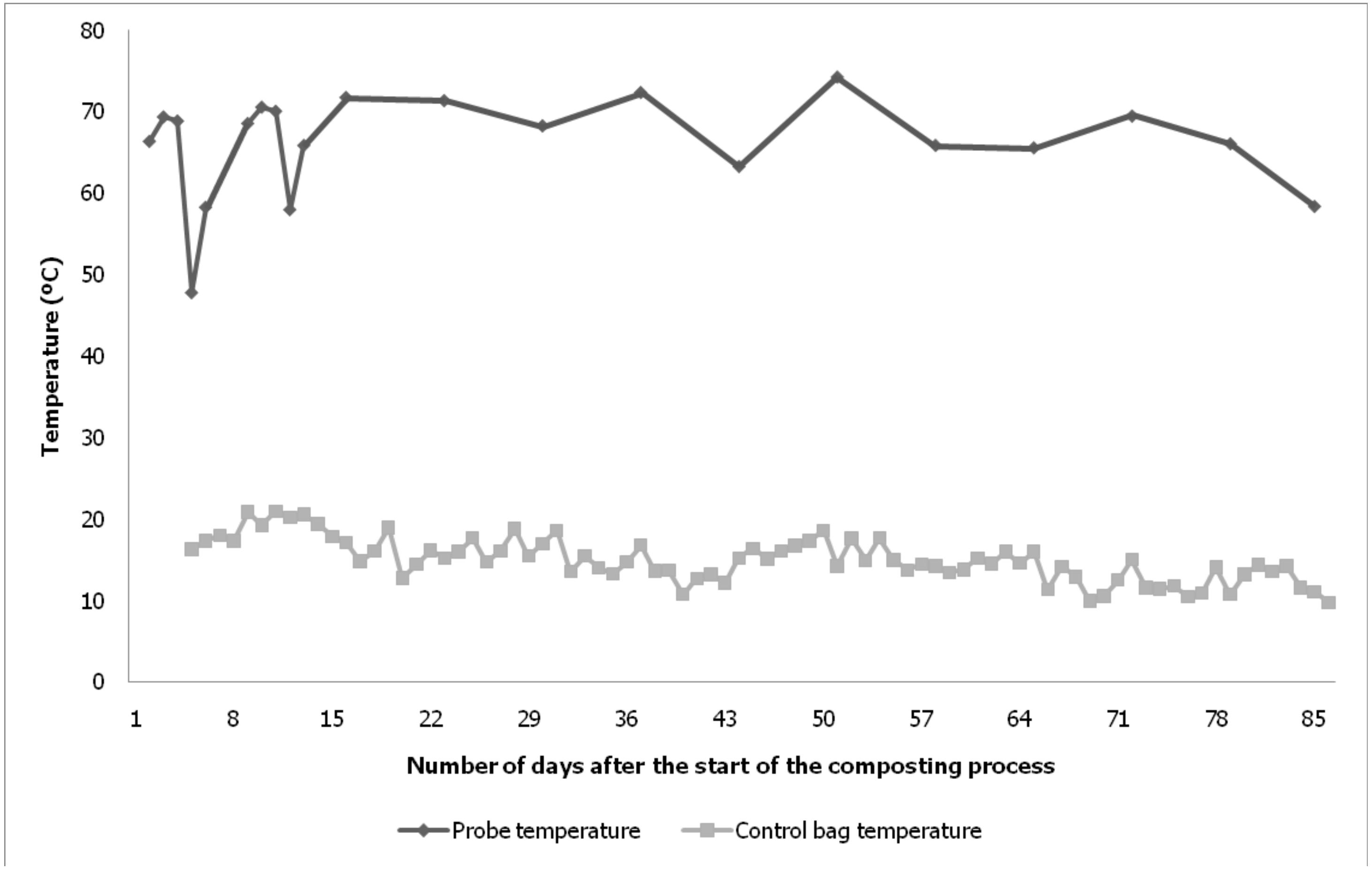

Figure 2. Temperatures recorded during the composting process by the data logger in the control bag (situated outdoors at air ambient temperatures and by temperature probe. Probe temperatures were averages based on readings taken from a depth of 1.5 m at four points throughout the compost windrow. Readings were taken daily during the sanitisation phase of composting, and weekly thereafter.

4.0 Discussion

The findings of this work are applicable to general UK commercial composting practice since it was carried out at a PAS100 accredited commercial composting site, following the standard operating procedures for production of its compost. The composting process used in this work was exactly the same as for normal commercial practice at the site concerned (in terms of the feedstock used, the dimensions of the windrow and the nature of the processes and monitoring involved).

Despite assurances from the manufacturers, the temperature logging devices were clearly not fit for purpose. These temperature logging devices were considerably more expensive than those used in previous trial work carried out by the authors, and were considered by both the manufacturers and the research team to be appropriate for this trial. As the entire experiment was complete and much of the analysis conducted before the data loggers could be retrieved as planned from the compost windrow in order to download the data, the failure of the loggers was discovered too late for remedial action to be taken.

The monitoring required under the standard operating procedures of the site used for this work showed that both compost temperatures and moisture content remained within the stated parameters required for critical control points to be satisfactorily achieved. That is, the moisture content remained within 40 – 65% throughout the duration of the trial and the temperatures within the windrow were in excess of 65°C for seven (not necessarily consecutive days).

The limit of detection for coniine was estimated at 0.1 mg/kg dry weight. An equivalent limit of detection for coniceine could not be determined as a reference standard was not available. As the structures of the two compounds are very similar it is highly likely that their ionisation process in the LC-ToF-MS and hence the limit of detection would be almost identical.

The mean concentrations of coniine measured in the composted and un-composted hemlock tissue (by LC-ToF-MS) both generally decreased over time, although concentrations were higher in plant material tested after 53 and 65 days than they were at the beginning or the end of the testing regime. No coniine was measured in the samples of hemlock retrieved from the compost windrow after 27 days of composting. It was decided to discard this data as an outlier on the advice of the chemist in the lab (Colin Crews, team leader toxicants and contaminants processing team, FERA, personal communication). There was not sufficient sample material to permit re-testing on that occasion.

Detectable concentrations of coniceine were measured only in hemlock material sampled 53 days after the start of the composting process, whether the plant material had been composted or not. As the detection limits for coniine and coniceine are highly likely to be similar, it is safe to conclude that coniceine levels were genuinely low throughout of the experiment (in comparison with coniine). The relationships between coniine and coniceine are little understood and the relative levels reported might be due to the maturity stage of the plants and/or affected by transformations during the composting process which are as yet unknown. Ratios of coniine and coniceine have been shown in the past to vary between different plant parts, and between plants collected from different sites (Lopez *et al*, 1998).

From the results obtained in this work, it appeared that concentrations of both coniine and coniceine increased in hemlock tissue as the plant material began to degrade, whether the plant material was being composted or was simply degrading naturally under ambient environmental conditions. It has been shown that the toxicity of cut/dying yew tissue increases as the fresh material dries and the toxins present become more concentrated in the material (see below), but the authors could find no documented evidence that the same happens with hemlock.

In practical terms, it was clear that although the composting process did not accelerate the degradation of toxins in the hemlock tissue, the lengthy nature of the composting process ensured that considerable degradation of the toxins present did occur during composting (there was less than 1 mg coniine/kg of plant tissue compared to 8.1 mg coniine/kg in the control sample at the first sampling event, and no coniceine present by the end of the 20 week composting process). The composting process at Fife Council's site is, however, longer than the average PAS100 composting process. There was still just over 4 mg coniine/kg (and no coniceine) present in the hemlock tissue after 12 weeks composting, which is a more usual length for a PAS100 accredited composting process.

Given that degradation of the toxins present in hemlock seems to happen equally well in dead plant material placed at ambient environmental conditions outside a composting process, it is likely that degradation starts as soon as the material is cut. Since green waste is usually stored in parks and gardens, and then on the composting site for up to several weeks before shredding, degradation of the toxins in any hemlock present in compost feedstock is likely to have been occurring during that storage period. Any storage period is therefore likely to reduce still further the concentrations of toxins present in the hemlock tissue at the end of the composting process.

Hemlock, which is a native wild plant that favours moist soils, has been recorded in several areas in Scotland, but it is not at all common. It is more common in England, though local authorities there often try to control it where it grows in public places, since it is very poisonous to humans. It has never been recorded in a feedstock pile on any commercial composting site quantities (A Litterick, personal experience from extensive work experience on commercial composting sites) and it is thought highly unlikely ever to be present in large quantities in feedstock. The toxins present in hemlock are likely to partially degrade prior to the start of any composting process in which they are included. This, combined with the degree of degradation which was demonstrated during this work, along with the fact that if present, hemlock is likely to be highly diluted within the feedstock pile means that the toxins present in hemlock are expected to pose a negligible risk to livestock grazing on compost-treated fields.

Concentrations of taxines A and B (as determined by LC-ToF-MS) in samples of yew material which had been buried in the compost windrow dropped over time until they were no longer detectable after 63 days of composting. In contrast, concentrations of taxines A and B in the control sample of yew clippings, which had been held under ambient environmental conditions still had mass spectrometry peak areas of almost 600 000 and over 1 000 000 respectively after 63 days. Degradation of the toxins was complete in yew clippings which had been composted over a period of 63 days, but it was very slow where the clippings were not subjected to composting conditions.

Yew foliage is reported to be more poisonous when wilted or dried than it is when fresh (Williamson, 1978), and it has also been reported that lethal poisoning occurred in sheep when they had access to garden refuse containing yew clippings (Zettl and Brömel, 1986). These findings suggest that the toxicity of cut yew increases as the fresh material dries and the taxoids become more concentrated in the material. There did appear to be an increase in the concentration of taxines A and B after cutting in the material in the control bag (not in the samples which were being composted. However, it is not possible to tell from this work whether the increases in taxine concentrations are genuine, since only a single sample was taken from the control bag on each sampling occasion.

The results from yew toxin determinations in this work broadly agree with those of Potter and Pitman (1995) and the advice of Defra (2005), in that they have shown that the composting of poisonous plant material results in degradation of plant toxins. However the method of composting used in this study (i.e. PAS100 accredited) differed from that reported in other studies, and toxin degradation in the other studies may have been due to different factors associated with non-PAS100 accredited composting than those discussed here, such as partial anaerobic decomposition. The speed of degradation of the toxins in yew may depend on factors such as temperature and relative humidity, though it is possible that micro-organisms are also involved. An analysis of the causes and mechanisms of taxoid degradation was outside the scope of this work, which was simply designed to determine whether complete degradation of the taxoids present occurred within the timeframe of the composting process.

Yew is a native evergreen conifer, which is relatively common in parks, churchyards and large gardens, but rather less common in smaller domestic gardens. It has occasionally been recorded in feedstock piles on commercial composting sites, though never in large quantities (A Litterick, personal experience from extensive work on commercial composting sites) and it is thought unlikely to be present in large quantities in feedstock mixes, although this is not significant when considered in the context of the findings of this work. On the basis of this work, the toxins present in yew are likely to completely degrade during a 9 week composting process. This, along with the fact that if present, yew is likely to be highly diluted within the feedstock pile means that the toxins present in yew are expected to pose a negligible risk to livestock grazing on compost-treated fields.

The results obtained from this project have shown that the toxins present in two species of poisonous plant degrade completely during a typical PAS100 composting process, although whereas degradation of one group of compounds appeared to be actively promoted by composting conditions, the degradation timespan of the other group merely coincide with the duration of a typical compost production cycle and is not promoted by composting conditions. The toxins present in yew and hemlock are both alkaloids, yet they behave very differently when the plants in which they are contained are composted. Ideally many more samples would have been tested (both from within the windrow and from the controls), but the high cost of testing limited the number of samples tested in this instance. An analysis of the causes and mechanisms of coniine, coniceine and taxine degradation was outside the scope of this work, which was simply designed to determine whether complete degradation of the toxins present occurred within the timeframe of the composting process.

5.0 Implications for growers/farmers, and other compost users

- Toxins present in yew plant material, which was composted in a typical PAS100 outdoor windrow composting process were completely degraded after 65 days of composting.
- The concentration of coniine present in hemlock plant material dropped considerably during a 20 week composting period, whether the material was composted or maintained under ambient environmental conditions.
- The concentration of coniceine present in hemlock plant material dropped over time until it was no longer detectable in plant material which had been composted, or in plant material which was maintained under ambient environmental conditions.
- The amount of yew and hemlock material present in typical green waste compost windrows is likely to be very low. Hemlock in particular is a native wild plant which is unlikely to occur in gardens. The dilution of any yew and hemlock material which does come into PAS100 composting sites is likely to be very great. For these reasons, there is no need to actively exclude yew and hemlock from feedstock accepted on to UK PAS100 accredited composting sites.
- The risk to livestock from coniine and taxoid poisoning through grazing grassland top-dressed (or otherwise treated) with PAS100 green waste composts is likely to be negligible.

6.0 Recommendations for future work

To better understand the representative nature of this research it would be instructive to determine toxin concentrations in yew and hemlock at different times of year, in order to enable selection of plant species and plant parts containing high concentrations of toxins for further studies of the degradation of toxins present. Before similar work is conducted in future, detailed trials should be carried out to determine ways of ensuring that the data loggers chosen will last the duration of the composting process. It may be possible to seal the loggers in watertight, heatproof covers, in order to prevent moisture from damaging them.

7.0 Acknowledgements

SAC thank WRAP for funding this work. SAC also thank Fife Council staff for providing a compost windrow and considerable practical assistance free of charge for this project.

References

AfOR (Association for Organics Recycling, 2009). Market survey of the UK organics recycling industry – 2007/08. WRAP, Banbury, UK. ISBN: 0-9547797-5-4.

Crews, C., Driffield, M., Berthiller, P. and Krska, R. (2009) Loss of pyrrolizidine alkaloids on decomposition of ragwort (*Senecio jacobea*) as measured by LC-TOF-MS. *J. Agric. Food Chem.* 57(9): 3669-3673.

Dass, C. (2007) Time of flight mass spectrometry. pp 80-86 In: Fundamentals of Contemporary Mass Spectrometry. John Wiley & Sons, Inc., Chichester, UK.

Defra (2005) Guidance on the disposal options for common ragwort. Defra Publications, Admail 6000, London, UK.

Leete, E. and Adityachaudhury, N. (1967) Biosysnthesis of the hemlock alkaloids-II. The conversion of γ-coniciene to coniine and Ψ-conhydrine. *Phytochemistry.* 6: 219-223.

Lopez, T., Cid, M. and Bianchini, M. (1999) Biochemistry of hemlock (*Conium maculatum* L.) alkaloids and their acute and chronic toxicity in livestock. A review. *Toxicon*. 37: 841-865.

McMaster, M. (2005) Time of flight analyser. pp 79-81 In: LC/MS A Practical User's Guide. John Wiley & Sons, Inc., Chichester, UK.

Macaulay Research Institute (2009) Risk assessment of the use of PAS100 green composts in Scottish Livestock production. WRAP, Banbury, UK (In preparation).

Potter, D.M. and Pitman, R.M. (1995) The extraction and characterisation of carcinogens from bracken and the effect on composting. pp. 191-196 In: R.T. Smith and J. Taylor (Eds.) Bracken, an Environmental Issue. The University, Leeds, UK.

Wilson, C., Sauer, J. and Hooser, S. (2001) Taxines: a review of the mechanism and toxicity of yew (*Taxus spp.*) alkaloids. *Toxicon.* 39: 175-185.

Williamson, X. (1978) The Great Yew Forest - the Natural History of Kingsley Vale. Macmillan, London, UK.

WRAP (2009) The influence of outdoor windrow composting on the concentration of grayanotoxins in rhododendron leaves. Report on Project OAV021-012, WRAP, Banbury, UK.

Zettl, K. and Brömel, J. (1986) Poisoning of sheep and goats by yew and rhododendron clippings. *Praktische Tierarzt,* 67: 317-321.

www.wrap.org.uk/agriculture/