



# Fluorescence properties of some farm wastes: implications for water quality monitoring

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## Abstract

Some farm wastes have been analysed for their fluorescence properties using fluorescence excitation–matrix (EEM) spectroscopy. Farm wastes investigated were silage liquor, pig and cattle slurry, and sheep barn waste. All farm wastes exhibited high intensities of fluorescence that can be attributed to the protein tryptophan. Silage liquor was characterised by a very high fluorescence intensity and an initial tryptophan : fulvic-like fluorescence intensity ratio of  $>20$ . Cattle and pig slurries exhibited a lower tryptophan : fulvic-like fluorescence intensity ratio ( $\sim 2$ – $5$ ) and lower tryptophan fluorescence intensity, and tyrosine fluorescence was also observed. Sheep barn wastes had the lowest tryptophan : fulvic-like fluorescence intensity ratios ( $\sim 0.5$ – $4.0$ ). Farm waste samples were reanalysed under controlled temperature conditions over a period of 50 days after sampling, to investigate the stability of their fluorescence properties. For silage liquor, tryptophan : fulvic-like fluorescence intensity ratios were observed to decrease with time, and were associated with a decrease in tryptophan fluorescence intensity, suggestive of clostridia breakdown of protein. For slurry samples, tryptophan : fulvic-like fluorescence intensity exhibited a more variable time-evolution, and tryptophan fluorescence intensity increased through time; the more complex fluorescence signal is due to the relatively heterogeneous nature of the slurry. Sheep barn waste samples exhibited more stable tryptophan : fulvic-like fluorescence intensity ratio and tryptophan intensities, suggesting these samples were more stable due to their greater age and decomposition. The ratios of tryptophan : fulvic-like fluorescence intensity observed from the farm wastes investigated are significantly higher than those observed in the majority of river waters, suggesting that farm waste pollution events could leave a signature in river waters due to their distinctively high protein fluorescence intensity. © 2001 Elsevier Science Ltd. All rights reserved.

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## 1. Introduction

Pollution derived from agricultural activities increases with intensification of farming. In particular, livestock farming has increased such that ever more numbers of animals are reared on the same area of land. For example, total livestock numbers in the UK totalled 11.4 million cattle, 44.6 million sheep and 7.3 million pigs in 1999, a 34% increase from the 1961 total; similar increases have occurred throughout Europe [1]. These

increases have led to an associated increase in farm waste products (for example slurry, dirty water, silage liquor, manure), which have to be stored and/or disposed off in an environmentally appropriate manner. All of these organic waste products have an impact on the environment if released into river systems; discharge leads to the rapid growth of river micro-organisms which generate a high biological oxygen demand (BOD). Different wastes generate varying BOD levels; from 140,000 mg/l for milk; 30,000–80,000 mg/l for silage effluents, 10,000–30,000 mg/l for pig and cattle slurries and 1,000–5,000 mg/l for dirty water (parlour and yard washings) [2]. These values are between 1 and 3 orders of

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magnitude greater than the BOD impact of sewage. Adversely high BOD leads to a decrease in river oxygen levels, which can lead to the death of aquatic life. Khaleel et al. [3] demonstrated that highest river BOD values were associated with high stocking densities or direct discharge of effluents into streams. Foy and Kirk [4] demonstrated a correlation between stocking density with streamwater BOD, with large BOD peaks generated by silage liquor discharges and smaller peaks by slurry applications. The number of organic pollution incidents in England and Wales over the period 1996–1999 averaged 2200 per year [5]. The majority of serious pollution incidents arise from accidental point discharges of slurry and/or silage (for example from leaks in silage or slurry tanks), rather than from high BOD generated after periods of rainfall affecting both point and diffuse sources (for example slurry applications). A comprehensive review of the impacts of organic effluents can be found in [6].

Although several studies have investigated the impact of farm wastes on river BOD, to the authors' knowledge no research has been undertaken on their fluorescence properties. Fluorescence occurs when electrons contained within molecules firstly gain and then release energy. Electrons have energy associated with them. The energy at which the molecule is most stable is called the ground state. If one or more electrons in a molecule are raised to a higher energy level, in our case through the absorption of light, then the molecule is said to be in an excited state. However, molecules preferentially exist in their lowest energy state; therefore an excited molecule will lose energy with the electron falling back to the ground state. During fluorescence, the excess energy is lost in the form of light. Fluorescent organic matter is a ubiquitous constituent of the natural environment, it is derived from the decay of plant and animal matter, and is present in soil, river, marine and ground waters, and has undergone extensive research [7–10]. The most important fluorescence characteristic of organic matter is the number of consecutive conjugated double or triple bonds (i.e. double or triple bonds separated by a single bond). Electrons involved in double or triple bonds are held in  $\pi$  orbitals and can move easily between energy levels more easily than electrons in other orbitals. Aromatic rings (containing conjugated double bonds) fluoresce for the same reason. The content of substituent groups such as carboxyls and carbonyls (having double bonds) also increases the wavelengths of excitation and emission. Molecules containing oxygen or nitrogen atoms, having lone electron pairs, are capable of resonance, as are aromatic rings, and this also enhances fluorescence [11].

Molecular structures which generate the principle fluorescence centres in organic matter observed in the environment principally comprise two groups. Firstly, high molecular weight organic molecules which contain

aromatic compounds, and which have a high content of carboxylic groups and polycondensed aromatic and conjugated structures often described as humic-like and fulvic-like substances [7]. Secondly, aromatic protein fluorescence (predominantly tryptophan and tyrosine) from protein groupings within amino-acids [9,12]. Recent advances in fluorescence spectrophotometry permits the rapid (<10 min) collection of fluorescence data from water samples at high optical resolution (0.5 nm) and the generation of excitation and emission data in the form of excitation–emission matrices [13]. Based on previous research, Fig. 1 presents a fluorescence excitation–emission matrix (EEM) of a hypothetical water sample. The discrete locations of fluorescence intensity peaks can be ascribed to the different fluorophores (fulvic-like (A), humic-like (B), tyrosine (C) and tryptophan (D) described above. Each fluorescence peak may vary in fluorescence intensity, relative to each other depending on the organic matter source, whereas overall fluorescence intensity variations will reflect changes in concentration. Hence by determining both the ratio of fluorescence intensity of each fluorescence peak, together with the absolute intensity, it may be possible to differentiate different farm wastes.

Analysis of the fluorescence of farm wastes could, if they have distinctively different EEM properties such as distinctive ratios of fluorescence intensity of the different fluorescence centres, lead to a detection method for farm waste pollution. Here we use fluorescence EEM data for the first time to determine the EEM properties of some farm wastes under laboratory conditions. Silage liquor and slurry samples were chosen as the main wastes for investigation, as these are responsible for the majority of severe farm waste related water pollution incidents in the UK; 948 out of 2072 agricultural pollution incidents in 1999 were related to dairy and beef farming, and a further 280 cases were related to sheep and pig farms (Environment Agency, personal communication). Given successful differentiation of these different farm wastes from each other, the second stage of investigation will be to investigate river EEM signatures in both farm waste polluted and unpolluted rivers. Given the relatively rapid data collection time and small sample size needed (<5 ml), fluorescence EEM data has the potential to provide a quick analytical method for water quality monitoring to assess impact of farm pollution events on river systems from grab water samples, such as those obtained in England and Wales by the Environment Agency, the regulatory body for water quality.

## 2. Experimental

Silage liquor and pig slurry samples were collected from Cockle Park Farm, near Morpeth, NE England. Cockle Park is mixed arable/poultry/pig/cattle research

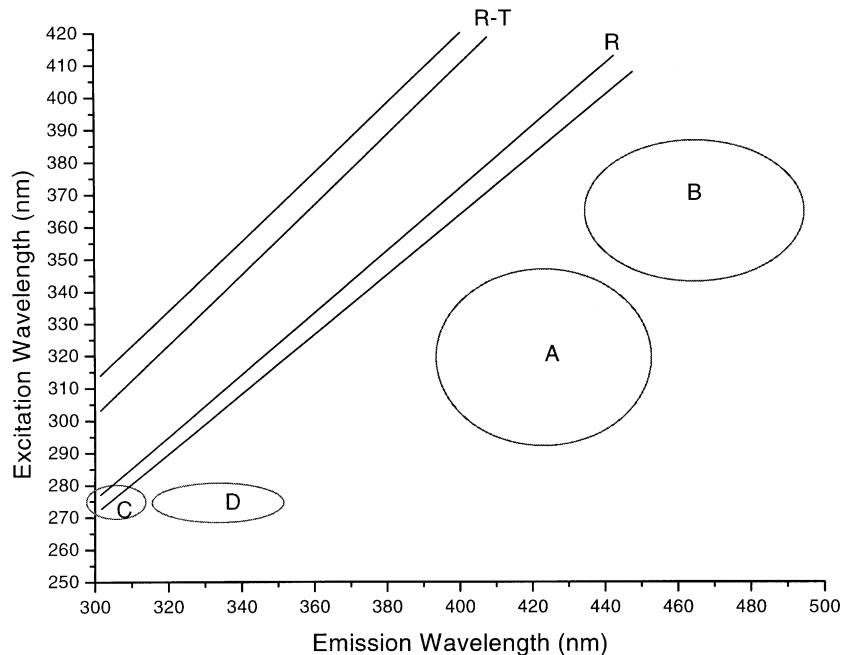


Fig. 1. Fluorescence EEM for a hypothetical water sample, to show all possible fluorescence centres observable in farm wastes. (A) Fulvic-like (B) Humic-like (C) Tyrosine (D) Tryptophan. Tryptophan fluorescence occurs at 275 nm excitation; 350 nm emission; tyrosine fluorescence at 275 nm excitation; 305 nm emission; fulvic-like fluorescence at 320–340 nm excitation; 410–430 nm emission; and humic-like fluorescence at 370–390 nm excitation; 460–480 nm emission. Note also the linear features of Rayleigh–Tyndall (R–T) scatter at  $\lambda_{ex} = \lambda_{em}$ , and the Raman line (R) of water. Note the closeness of the tryptophan and tyrosine fluorescence centres, and the location of tyrosine fluorescence at the same location as the Raman line.

farm for the University of Newcastle. Silage liquor samples were collected at 1–3 day intervals from the liquor tank during the first hay cut (May 2000). Pig slurry was collected from the slurry tank in June 2000. Cattle slurry was collected from a small dairy farm at Heddon in the Hill, near Newcastle, NE England, after yard cleaning on three occasions in June 2000. In addition, barn waste was collected from an upland sheep farm at Dalesfoot, near Kirkby Stephen, N England. Five samples of waste that comprised ~30% hay, 70% excrement was collected during muck spreading in April 2000. All farm waste samples were initially collected in 60 ml polypropylene bottles which had been precleaned in 10% HCl, Decon and distilled water. Samples were refrigerated upon return from the field, and first analyses for their fluorescence properties were undertaken within 24 h. Samples were not filtered, as we wished as far as possible interactions that would occur in the natural environment. Silage liquor and pig slurry samples had to be diluted by  $\times 1000$  to obtain a signal low enough to be measurable on the luminescence spectrophotometer, and to avoid inner filtering and pH effects [13]. Sheep barn waste samples were dissolved in 500 mg of distilled water to an approximate 40 mg/l (wet weight) concentration, and cattle slurry to an approximate 200 mg/l wet

weight concentration. Samples were not dry weighed as drying could alter the organic matter fluorescence. Concentrations were chosen as to optimise the signal:noise ratio on the spectrophotometer. Additionally, all samples had a pH of 5–7, thus pH variations did not significantly affect fluorescence properties. In order to investigate any changes in fluorescence properties with farm waste breakdown, samples were stored for up to 50 days under different temperature conditions. Samples were subdivided, with samples being stored in the dark at  $22 \pm 3^\circ\text{C}$  and at  $3 \pm 1^\circ\text{C}$ . Samples were reanalysed at regular (approximately weekly) intervals over the 50 day period after collection.

Fluorescence measurements were undertaken using a Perkin-Elmer LS-50B luminescence spectrometer. The spectrometer used a xenon excitation source, and slits were set to 5 nm for both excitation and emission. To obtain fluorescence EEMs, excitation wavelengths were incremented from 200 to 400 nm at 5 nm steps; for each excitation wavelength the emission was detected from 250 to 500 nm at 0.5 nm steps. Scan speed was set at 1500 nm/min, generating an EEM in ~15 min. For each water sample, the fluorescence was measured as the maximum intensity at an excitation–emission wavelength pair. Analyses were performed at a constant

laboratory temperature of  $22 \pm 3^\circ\text{C}$ , and blank water scans were run every 5–15 analyses using a sealed distilled water cell. The Raman peak of water at 348 nm was used as a test for machine stability and to permit inter-laboratory comparison. Raman emission at 395 nm averaged  $18.7 \pm 1.0$  intensity units ( $n = 147$ ), with no drift through the analytical period. Duplicate samples demonstrated that the wavelength of peak humic and fulvic-like fluorescence was reproduced within  $\pm 3$  nm and protein fluorescence  $\pm 5$  nm; fluorescence intensities replicated within  $\pm 5\%$  and  $\pm 15\%$  respectively. In addition, the stability of the Raman peak was assessed for a 10 min period at the start of each day of data collection and sample collection occurred only when the signal:noise ratio of the spectrometer was greater than 500:1.

### 3. Results and interpretation

Fig. 2 presents typical EEM data for each of the farm wastes analysed for the initial analysis (within 24 h of collection). It is apparent that each of the wastes at this stage has distinctive and different fluorescence properties. The sheep barn waste sample (Fig. 2a) has a low tryptophan:fulvic-like fluorescence intensity ratio of  $\sim 2:1$ , low fluorescence intensity (40–100 intensity units

at 40 mg/l solution) and no tyrosine fluorescence observed. Pig and cattle slurries (Figs. 2b and d) are similar to each other; tryptophan:fulvic-like fluorescence intensity ratio is  $\sim 4:1$ , fluorescence intensities are less than that of the silage liquor ( $\sim 50$  units after a  $\times 1000$  dilution for pig slurry and 60–100 units at 200 mg/l for cattle slurry). These samples are distinguished by the presence of tyrosine fluorescence at a similar intensity to that of tryptophan. Finally, silage liquor (Fig. 2c) has very strong tryptophan fluorescence, with a tryptophan:fulvic-like fluorescence intensity ratio of 25:1, and very intense tryptophan fluorescence (400–800 intensity units after a  $\times 1000$  dilution); no tyrosine fluorescence was observed. The initial fluorescence EEM results are summarised in Table 1, together with the mean tryptophan:fulvic-like fluorescence intensity ratio observed after 50 days, as well as typical results observed in river waters.

#### 3.1. Silage liquor

The fluorescence EEM data over the 50 days subsequent to sample collection is presented in Fig. 3, with data simplified to show just the tryptophan:fulvic-like fluorescence intensity ratio. Results demonstrate that the mean fluorescence intensity ratio decreases with

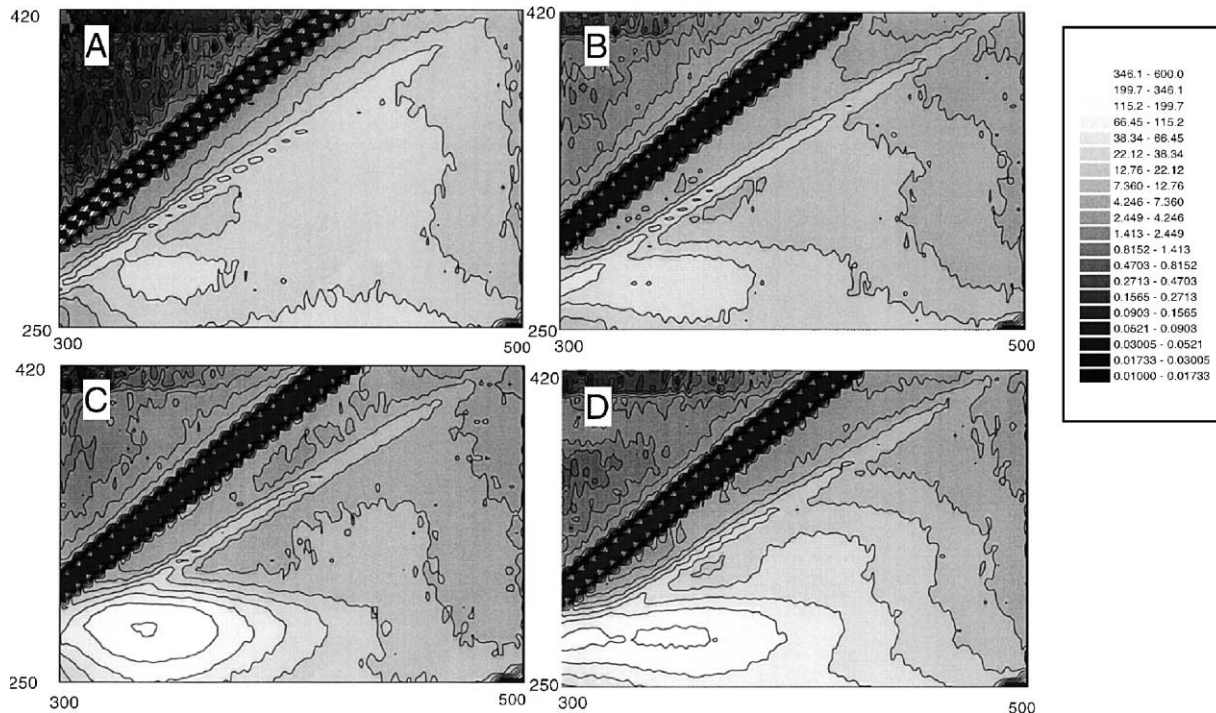


Fig. 2. Fluorescence EEMs for each of the four farm wastes investigated and analysed within 24 h of collection. (A) Sheep Barn Waste (B) Cattle Slurry (C) Silage Liquor (D) Pig Slurry. Note the logarithmic intensity scale and the difficulty in separating tyrosine and tryptophan fluorescence centres.

Table 1

Summary of the fluorescence properties (fluorescence ratio of tryptophan intensity/fulvic-like intensity; tryptophan fluorescence intensity and presence/absence of tyrosine fluorescence) of farm wastes and river water samples

Sample type	Concentration <sup>a</sup>		Initial fluorescence (within 24 h)			Final fluorescence (after 50 days)		
			Tryptophan: fulvic-like intensity ratio	Tryptophan intensity	Tryptophan fluorescence	Tryptophan: fulvic-like intensity ratio	Tryptophan intensity	Tyrosine fluorescence
Silage liquor	22±3°C 3±1°C	1 mg/l	21.5±6.9	571±257	No	8.8±1.5	309±110	No
			25.1±4.3	765±150	No	17.8±2.9	374±43	No
Pig slurry	22±3°C 3±1°C	1 mg/l	4.2	200	Yes	3.4	150	No
			4.7	260	Yes	4.1	150	No
Cattle slurry	22±3°C 3±1°C	~200 mg/l	3.3±0.2	77±18	Yes	3.7±1.7	98±30	No
			3.5±0.5	85±12	Yes	5.9±1.4	98±8	No
Sheep barn waste	22±3°C 3±1°C	~40 mg/l	1.8±0.8	67±69	No	1.5±0.6	88±14	No
			2.1±0.9	72±41	No	2.2±0.8	88±25	No
River samples	1–15 mg/l		0.4±0.4	28±28	No			

<sup>a</sup>Concentrations are based on wet weights of initial samples. River water concentration is that quoted in [14]. Fluorescence intensities are standardised to a Raman blank of 20.0 intensity units.

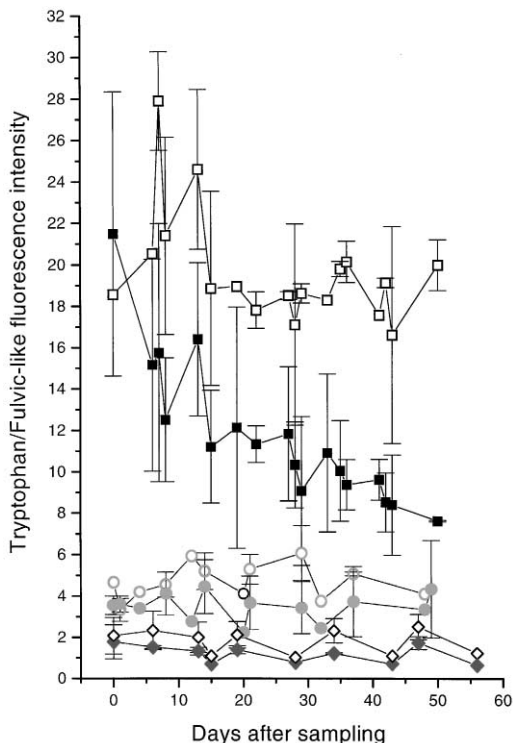


Fig. 3. Fluorescence of silage liquor (squares), pig and cattle slurry (circles) and sheep barn waste (diamonds) over a 50 day period, samples stored in sealed containers at 22±3°C (solid) and 3±1°C (hollow). Error bars are the standard deviation of six (silage liquor) and four (slurry and barn waste) samples.

time from ~25:1 to a ratio of ~9:1. The rate of decrease is slower at lower temperatures. Associated with this decreased protein fluorescence is a decrease in tryptophan fluorescence intensity and a stable fulvic-like fluorescence intensity, suggesting that protein groups are being broken down into non-fluorescent structures.

Silage liquor is formed through the spontaneous fermentation of (in our study) a grass crop, through the action of lactic-acid bacteria under anaerobic conditions [15]. Silage liquor comprises a high proportion water-soluble carbohydrates, fibre, lactic acid and protein [15]; most of the protein is enzymic in nature and includes fluorescent tryptophan and tyrosine. The amino-acid content of protein does not vary greatly with species [16], but is affected by stage of growth (declining from approximately 200 to 30 g/kg dry mass with increasing time of first cut [17] and fertiliser use. One undesirable micro-organism present during silage fermentation is clostridia; these bacteria ferment proteins under anaerobic conditions. These biochemical characteristics of silage liquor explain our fluorescence results. The very high protein:fulvic-like fluorescence intensity ratio reflects the biochemical structure of grass, and the decrease in protein fluorescence through time most likely reflects the action of clostridia. Samples stored at low temperature experienced the least change in protein:fulvic-like fluorescence intensity ratio and probably reflects the fact that clostridia growth will be inhibited at low temperatures and hence catabolism of aromatic amino-acids will be inhibited. Although both tyrosine and tryptophan are observed in both silage

liquor and grass crops, we do not observe tyrosine fluorescence here. Tyrosine fluorescence is relatively difficult to measure, given its relatively low fluorescence efficiency when compared to tryptophan, due to interference caused by its close location to the tryptophan fluorescence centre, energy transfer between tryrosine and tryptophan fluorophores, and the interference of the Raman line of water [12]. Given the interference effects described above, although tyrosine is probably present, it is at a low enough concentration such that its fluorescence is not observed.

### 3.2. Pig and cattle slurry

Pig and cattle slurry EEM data are presented in Fig. 3; showing changes in the tryptophan:fulvic-like fluorescence intensity ratio. Fig. 3 demonstrates that the initial tryptophan:fulvic-like fluorescence intensity ratio ranged from 2.5 to 5.0. All slurry samples exhibited different time evolutions of this ratio, although for all samples there was an increase in fluorescence intensity through time. The total range of tryptophan:fulvic-like fluorescence intensity ratio varied from  $\sim 2$  to  $\sim 6$ ; samples which were stored at low temperature exhibited more detail in tryptophan:fulvic-like fluorescence intensity evolution. Pig slurry samples behaved in a similar manner to cattle slurry, although overall tryptophan fluorescence intensity was much higher.

Pig and cattle slurry samples also exhibited a weak fluorescence peak that is associated with tyrosine (Fig. 2). After correcting for interferences from the water Raman line and tryptophan fluorescence, the initial samples exhibited a tyrosine:tryptophan fluorescence intensity ratio of  $\sim 1$ . In both the pig and cattle slurry samples, the tyrosine fluorescence peak rapidly declined and after more than one day was not distinguishable above background fluorescence. The slurry samples therefore exhibit subtle differences from the silage liquor, with an increase in tryptophan fluorescence through time, and a more complex time-evolution of tryptophan:fulvic-like fluorescence intensity ratios. These differences can be attributed to slurry being a more complex and heterogeneous organic source. Animal species, physiological state and diet all affect the composition of excreted material [18], and this would in turn affect their fluorescence characteristics. Research has demonstrated that livestock faeces typically comprise 15–25% protein in wet manure (poultry, cattle and pigs [19]); within this  $< 3 \text{ g}/16 \text{ g}$  true protein N is tryptophan and tyrosine [19]. Hence protein fluorescence is expected. Experiments on the biodegradation of slurry by microbial populations have demonstrated an initial peak in respiration within 24 h, followed by a much lower but maintained respiration rate for at least 20 days [18]. This was interpreted as the initial degradation of the soluble fraction, followed by a

slower degradation of the less soluble material. Continued evolution of the fluorescence characteristics of our slurry sample after 50 days would agree with such microbial degradation of the slurry samples.

### 3.3. Sheep barn waste

Sheep barn waste EEM data are presented in Fig. 3, showing the ratio of tryptophan:fulvic-like fluorescence intensity. This ratio varies from 0.5 to 3.0, with a greater variability occurring between samples than between storage conditions, representing the natural variability of this farm waste. No systematic change in fluorescence ratio can be observed through time, presumably due to the fact that this waste has already had substantial time to decompose before being sampled. Similarly, no tyrosine fluorescence was observed, although this may have been present prior to sampling.

## 4. Conclusions

Fig. 3 and Table 1 demonstrate that silage liquor has a particularly intense fluorescence even at low concentrations and a distinctively high tryptophan to fulvic-like fluorescence intensity ratio. In terms of the monitoring of farm wastes, these characteristics suggest that silage liquor should be detectable if present in river and groundwaters. Pig slurry is similarly intense in tryptophan fluorescence and should therefore also be observable even when diluted in rivers. Cattle slurry and sheep barn waste samples had to be prepared at artificially high concentrations in comparison to river TOC, and so may be less easily detected when diluted. However concentrations similar to those used in our laboratory analyses could be observed during pollution events by, for example, a slurry store failure.

It is informative to compare the farm yard waste fluorescence with that of river water samples, to investigate whether they have significantly different fluorescent characteristics. River waters were sampled as opportunistic grab samples from rivers throughout the British Isles between January and August 2000, as well as from regular sampling at the Coalburn Experimental Catchment from October 1998 to August 2000 [20]. For these data sets, tryptophan:fulvic-like ratio equalled  $0.37 \pm 0.41$  ( $n = 242$ ) and tryptophan intensity  $28 \pm 28$  intensity units. These results suggest that farm wastes, which have higher tryptophan concentrations throughout the 50 day period from sampling, should be detectable from the background fluorescence of the river waters.

Further application of the fluorescence EEM technique to water pollution incidents is required. Also investigations into the fluorescence properties of a wider range of farm wastes, including silage liquor from grass

cuts at different stages in the growth cycle and different fertiliser applications, and from different silage crops, other slurries (e.g. chicken slurry) and wastes (e.g. milk). In addition, the use of lower fluorescence excitation wavelengths to separate tyrosine from the Raman line and to therefore better separate tryptophan and tyrosine fluorescence requires further investigation [12]. These are the focus of current research.

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