# FLASH MICROBIAL TOXICITY TEST AS MONITORING PARAMETER AT COMPOSTING: COMPARISON OF ECOTOXICITY LEVELS FOR DIFFERENT SUBSTRATES

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## 1 INTRODUCTION

International and national quality requirements define that compost shall not contain any environmentally harmful substances. Nevertheless, no established methods are available for measuring the toxicity of composted materials (Kapanen and Itävaara, 2001). Microbiological toxicity tests are gaining attention because they are fast, simple, sensitive and inexpensive. The bioluminescence-measuring test is based on the change in light emission by *Vibrio fischeri* when exposed to toxic chemicals. Tests can be performed with soil water eluates or with solid-phase samples (Flash method test) (Lappalainen et al., 1999). In Flash method, a kinetic measurement of the luminescence signal is started at the same time as the bacteria are added to the sediment sample. The advantage of this method is that it takes into account all the factors that effect the quenching of light: sample colour, adsorption of bacteria onto sediment particles and toxicity of the sample. This method has been previously used for measurement of soil and compost toxicity (Pollumaa et al 2000; Degli-Innocenti et al., 2001).

The aim of this work was to compare ecotoxicity of different materials (sewage sludge, plant wastes and municipal solid wastes) and their liquid extracts at several phases of composting by using the bioluminiscence Flash test method.

## 2 MATERIALS AND METHODS

### 2.1 Samples

Samples of sewage sludge (SS), plant wastes (PW) and municipal solid wastes (MSW) were collected at five composting phases (T1, initial; T2, termophile; T3, cooling; T4 maturation; T5, final compost). Solid samples (E0) and their aqueous extracts (1:5, solid:water) after 24h (E24) or 48h (E48) contact time were subjected to toxicity analysis.

### 2.2 Toxicity measurement of the samples

The total toxicity of the samples was measured with a BioTox™ kit (Aboatox Oy, Turku, Finland) according to the instructions of the kit. The kit contains freeze-dried *V. fischeri* (strain NRRL-B-11177) and reagents for toxicity testing according to ISO 11348-3. Samples were diluted (1:5) with sample diluent (NaCl 2%) and pH adjusted to 6.5-7.5 with NaOH or HCl when required. Sample diluent was also used as a control sample.

Bioluminiscence of *V. fischeri* was measured in a Luminoskan Ascent microplate luminometer (Thermo-Electron Co., Vantaa, Finland) at 20ºC (Lappalainen et al., 1999). This instrument is an automated luminometer capable of dispensing bacteria reagent, mixing and measuring the samples simultaneously. The testing was performed in 96-well microplate. An aliquot of 100 µl of the sample was transferred to each well, after which 100µl of the bacterial suspension was automatically dispensed into the sample. The light signal was recorded 20 times/s for 30 s after dispensing. The peak value of luminescence was obtained within the first 5 s and it was followed by a reduction in the case of toxicity of the sample. On the other hand, no bioluminescence decrease was recorded in the absence of toxicity. The luminometer was controlled by Ascent Software Version 2.4.1 (Thermo-Electron Co., Vantaa, Finland).

### 2.3 Data analysis

The results were presented as percentages of inhibition in light production. Inhibition percentage was calculated as the ratio of the maximum (peak) light production (0–5 s) against the light production after 30 s exposure time according to Eqs. (1) and (2):

$$KF=IC_{30}/IC_0 \quad (1)$$

$$\%Inhib=100-(IT_{30}/(KF \times IT_0)) \times 100 \quad (2)$$

where KF is the correction factor, $IC_{30}$ is the luminescence intensity of the control sample after 30s contact time (mV), $IC_0$ is the peak luminescence of the control sample (mV), $IT_{30}$ is the luminescence intensity of the test sample after 30s contact time (mV) and $IT_0$ is the peak luminescence of the test sample (mV).

The toxicity of the samples was also estimated as $EC_{50}$ values on the basis of dose-response curves. $EC_{50}$ represents the concentration of the tested substance when the amount of light produced by *V. fischeri* is reduced to one half. The dilution series for $EC_{50}$ analysis were made with sample diluent until 1:32. Thirty-second $EC_{50}$ values were determined from concentration versus %Inhib curves by means of standard least-squares statistics. The linear correlation coefficient reached values between 0.89 and 0.99.

## 3 RESULTS AND DISCUSSION

### 3.1 Toxicity of raw materials

The kinetic responses of the control sample and samples at time 1 (initial) are shown in Figure 1. The curve shapes of the control and PW and SS samples were clearly similar, showing a typical profile of non-toxic samples. In these samples the peak value of the luminescence was attained rapidly (2s) after dispensing the *V. fischeri* suspension onto the sample, and the luminescence level stayed fairly constant during the 30 s exposure time. The luminiscence signal of the control sample was higher throughout the measurement. This was due to colour and solid particles interferences that reduced the luminiscence. During data processing these interferences were corrected because samples were used as a reference themselves and the light output was recorded immediately after all the bacteria contact the sample. In MSW and PW 24h aqueous extracts (E24h) samples, luminiscence decreased after 2s contact time because of their toxic effect on *V. fischeri*. In all samples toxicity was higher as aqueous extraction time was longer, as can be seen for the lower light output in E24h and E48h extract samples.

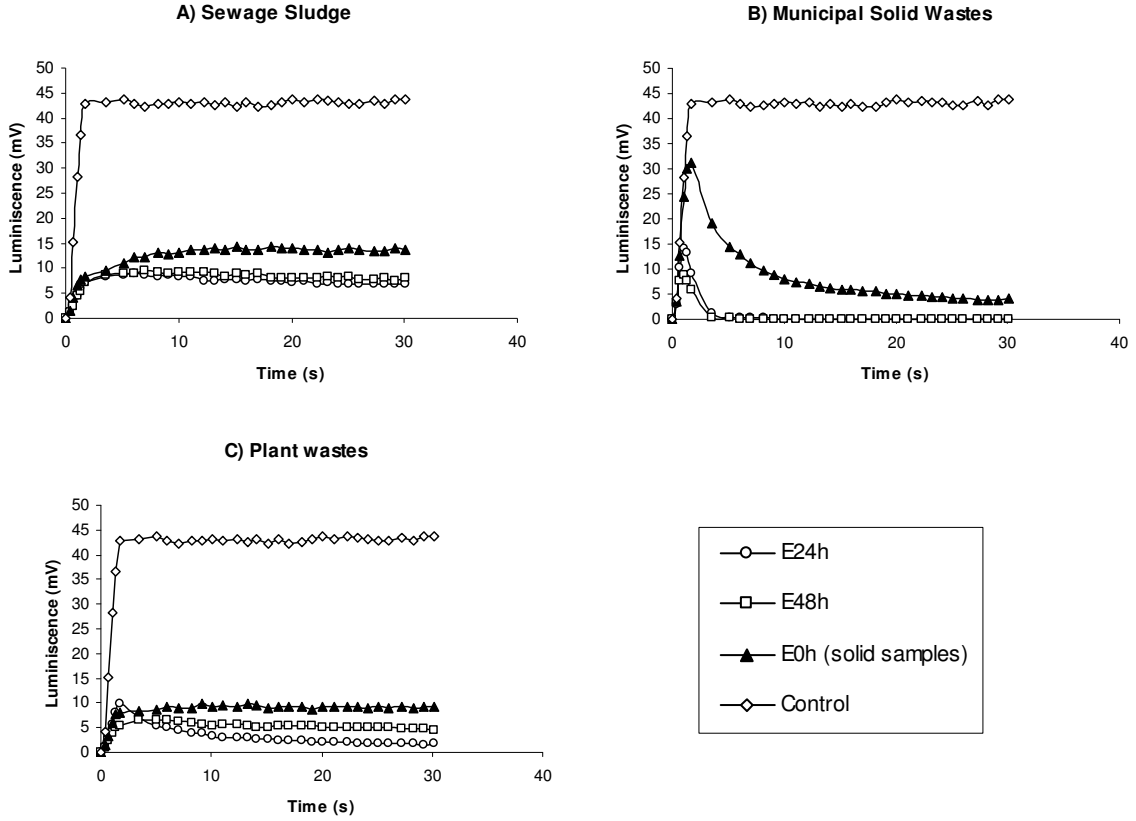


FIGURE 1 **Kinetic photobacterial measurement (30 s) of the control sample (NaCl 2%) (◊), and solid (▲) and 24h (○) and 48h (□) aqueous extracts of initial samples (T1) of (A) Sewage sludge (SS) (B) Municipal solid wastes (MSW) and (C) Plant wastes (PW).**

## 3.2 Evolution of toxicity during composting

At the beginning of composting, MSW samples showed higher toxicity levels (higher % inhibition) than PW and SS samples (Figure 2). For nearly all samples toxicity was increased as water extraction time was more prolonged. Hence, 48h aqueous extracts caused a more noticeable decrease in luminiscence than 24h or solid samples. Toxicity decreased during composting time for all samples analysed. This decrease was more pronounced in samples with higher initial toxic values such as MSW or 24h-PW extract. In these samples toxicity decrease mainly occurred after cooling composting phase. All mature compost had similar toxic values with luminiscence inhibition percentages below 20%.

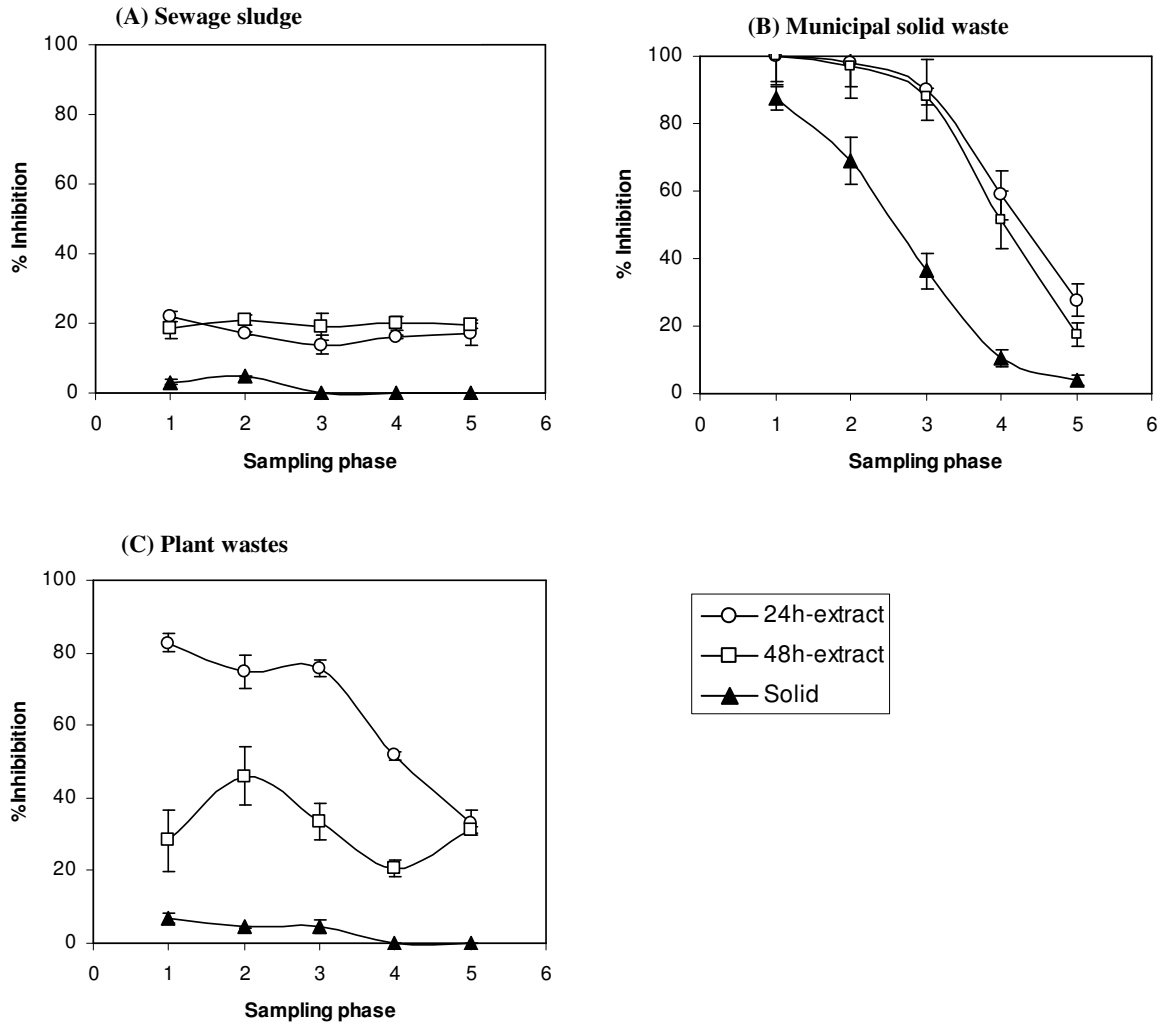


FIGURE 2 **Inhibition of *V. fisheri* luminiscence in the Flash test (% Inhibition) with solid compost (▲) and 24h (○) and 48h (□) aqueous extracts of (A) sewage sludge, (B) municipal solid wastes and (C) plant wastes at different stages of composting: 1, initial; 2, termophile; 3 cooling; 4 maturation; 5, final compost. Results are average values±SD of three replicates.**

Table 1 shows $EC_{50}$ values of samples analysed. Toxic effect was only exhibited by 24h aqueous extracts from PW and MSW samples. As noticed previously, toxicity decreased as composting proceeded and, as a consequence, final compost were non-toxic. The unique deviation of this trend was observed in 24h aqueous extracts from plant wastes whose $EC_{50}$ was higher (lower concentration decreased luminiscence) at more prolonged composting time up to maturation phase, but even for these samples 24h extracts from final compost were non-toxic.

TABLE 1 **$EC_{50}$ values of tested samples in *V. fischeri* Flash Assay after 30 s exposure. Values are % (v/v) of samples that lead to a decrease of 50% of luminiscence. nt: no toxic effect or low inhibition at the maximum concentration tested.**

| Sample | Composting phase | $EC_{50}$(%) | | |
|---|---|---|---|---|
| | | Solid | 24h extract | 48h extract |
| Plant wastes | 1 | nt | 43 | nt |
| | 2 | nt | 35 | nt |
| | 3 | nt | 33 | nt |
| | 4 | nt | 115 | nt |
| | 5 | nt | nt | nt |
| Municipal Solid wastes | 1 | 61 | 33 | 28 |
| | 2 | 84 | 39 | 38 |
| | 3 | nt | 46 | 42 |
| | 4 | nt | 99 | 97 |
| | 5 | nt | nt | nt |
| Sewage Sludge | 1 | nt | nt | nt |
| | 2 | nt | nt | nt |
| | 3 | nt | nt | nt |
| | 4 | nt | nt | nt |
| | 5 | nt | nt | nt |

## 4 CONCLUSIONS

In the present work the Flash test has been applied to verify the ecotoxicity of extracts from three raw materials during composting. The results of this study suggest that the Flash test can be reliably used for ecotoxicological analysis of these materials.

Municipal solid wastes samples had toxic values higher than plant wastes or sewage sludge. Even if the initial samples for composting have distinct toxic potential, composting act as an efficient method to reduce toxicity of organic materials because of stabilization processes. Mature compost had a negligible or null ecotoxic effect irrespective of raw materials used (MSW, PW or SS). However, attention should be paid to lixiviates generated from composting piles. This work demonstrated that aqueous extracts from compost samples might have a higher toxicity level than the solid samples. Consequently, uncontrolled lixiviates may contaminate other niches.

## ACKNOWLEDGEMENTS

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