Testing the genotoxicity of composted sewage sludge using the direct contact *Vicia faba-root* micronucleus test

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ABSTRACT

The sewage sludge induces severe environmental problems in Morroco. Because, composting has been proposed to mitigate the situation, it is necessary to assess the genotoxicity of composts. The present study focuses on the assessment of the genotoxicity of compost obtained by composting sewage sludge and green waste. The micronucleus reduction in vicia faba root by direct contact exposure to a solid matrix test was used to evaluate the genotoxicity of compost. The direct contact method was applied at 30% dilution of compost obtained by mixing compost with the standard LUFA soil for three stages of composting. Maleic hydrazide was used as a positive control and LUFA standard soil was used as a negative control in all direct contact tests. Results show that the tested sewage sludge at 30% dilution increased the mitotic index (MI), and causes a significant decrease in micronucleus frequency. During sludge composting, after the maturation phase, the reduction in micronucleus frequency reached 55%. Composting could be suggested as a detoxification process of sludge after a sufficient time of treatment ensuring a safe end product.

Keywords: Genotoxicity, composting, sewage sludge and Vicia faba

1- INTRODUCTION

Sewage sludge is a valuable by-product in wastewater plants, containing nutrients like nitrogen, phosphorus, and potassium which are essential for plant growth. It is a slow-release fertilizer and its organic material can improve soil quality. However, these sludge may also contain toxic elements (metal and organic micro-pollutants), limiting their recycling (Amir, 2005; Jouraiphy, 2005). Composting could be suggested as a detoxification process of sludge that can reduce pathogens and the organic contaminants in the sludge.

In the present work, the Vicia faba root-micronucleus assay was chosen to assess genotoxicity of sewage sludge compost.

2- MATERIALS AND METHODS

2.1. Test of composting

Raw sewage sludge produced in the Wastewater Treatment Plant of Marrakech (Morocco) was composted in a pile of 3.5 m^3, mixed with green waste in equal volumetric proportions. Composting evolution was monitored for a period of 210 days, and the mixture was turned over every 15 days to ensure aerobic conditions. The microbial activity was followed daily by measuring the temperature in the compost. Table 1 shows the main physico-chemical properties of raw materials. To obtain the representative samples and results of analysis, numerous samples from various point of the compost pile were collected and mixed thoroughly at various stage of composting:
The selected times of sampling were (initial mixture, after 15, 30, 60, 90, 120 and 210). The samples were kept deep frozen until the time of analysis.

Table 1: Physico-chemical properties of Raw materials

Settings	sewage sludge	Green waste
humidity (%)	64.8	64
pH	7.9	6
NTK (%)	3.14	1.5
COT (%)	31.8	52.8
C/N	28.3	35.2
Ashes rate (%)	45	8.33

2.2. Compost parameters

Some physico-chemical characteristics of the composts at different stages of treatment are reported in Table 2: The pH is determined on a suspension sample - water (10g / 15 ml), the Total Organic Carbon (C. org) is measured according to ANNE method, the total nitrogen (N.tot) by Kjeldahl method, The rate of decomposition is calculated after ignition of the dry sample at 550°C (16 h).

2.3.Genotoxicity assessment by direct contact

The direct contact method developped by Marcat_ Romain CE et al (2009) was used for assessment and the evaluation of compost quality. The direct contact method was applied to a concentration of compost 30% obtained by mixing compost with the standard LUFA soil. Roots were collected after a 2-d exposure. Controls used LUFA soil wetted with distilled water (NC) or a 10_5 M solution of maleic hydrazide (PC).

3- RESULTS AND DISCUSSION

a. Evolution of the sludge and green waste mixture during composting process

Two phases of the composting process were recorded: a phase of stabilization (about 30 days duration), where temperature peaked at 67 °C after 3 days of processing; a phase of maturation (about 150 days duration), characterized by a temperature plateau at 35 °C.

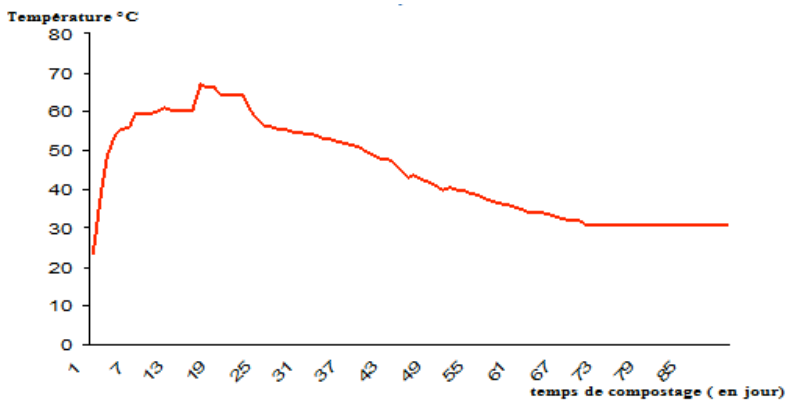

Fig1: Evolution of temperature during composting

The change in the C/N ratio from 16,3 to 11,5 and the increase of the amount of ash reflect microbial decomposition of organic matter and stabilisation during composting (Table 2). The increase of total nitrogen during composting was caused by the decrease of substrate carbon resulting from CO_2 loss (Soumaré ,2002 ; Zorpas ,2003).

Table 2: Physico-chemical properties of Raw materials and green plant waste at different times of composting (results expressed in dry basis).

Composting time	pH	Corg*	NTK	C/N	ashes	Dec%	P ass* mg/1g
T0d	7.55	34.37	2.11	16.28	34	-	1.42
T15d	7.43	32.4	2.04	15.68	0.4	22	2.12
T30d	7.84	31.53	2.45	12.86	43	31.71	1.9
T60d	8.93	29.4	2.32	12.64	50	48.48	2.07
T90d	7.97	27.46	2.41	11.53	52	52.44	2.23

b. Micronucleus test of compost

The results of the *Vicia faba* root-micronucleus test of sewage sludge compost obtained for three samples at different stages of composting (0 day, 120 days and 210 days) are presented in Fig. 2. The Mitotic Index (MI) was greater than 2% for all soil concentrations. Into the test soil, positive controls and mixtures at 120 days and 210 days led to an increase in mitotic index and a significantly decrease in Micronucleus (MN) frequency which reached 55% of the initial value (at 0 days).

(A)

(B)

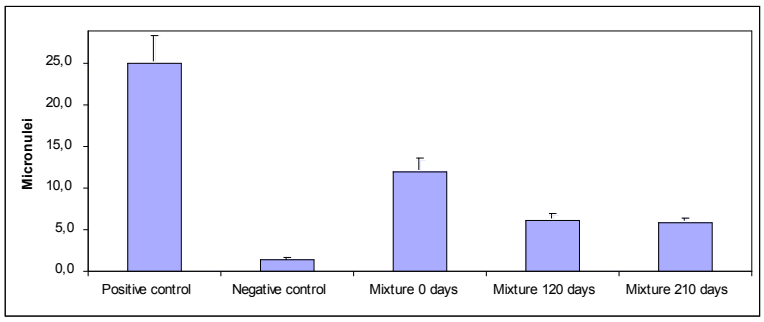

Fig 2: Mitotic index (A) and micronucleus frequency (B) values in *Vicia faba* roots exposed to samples of compost at different stages.

4. CONCLUSION

The direct contact method was applied at 30% dilution of compost obtained by mixing compost with the standard LUFA soil for three stages of composting. Maleic hydrazide was used as a positive control and LUFA standard soil was used as a negative control in all direct contact tests. Results show that the tested sewage sludge at 30% dilution increased the mitotic index (MI), and causes a significant decrease in micronucleus frequency. During sludge composting, after the maturation phase, the reduction in micronucleus frequency reached 55%. Composting could be suggested as a detoxification process of sludge after a sufficient time of treatment ensuring a safe end product.

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