

*Full Length Research Paper*

# Oil palm empty-fruit bunch application effects on the earthworm population and phenol contents under field conditions

D. T. Sabrina<sup>1</sup>, A. W. Gandahi<sup>2,3</sup>, M. M. Hanafi<sup>2\*</sup>, T. M. M. Mahmud<sup>4</sup> and A. A. Nor Azwady<sup>5</sup>

<sup>1</sup>Department of Land Management, Faculty of Agriculture, Universiti Putra Malaysia, 43400, Serdang Selangor, Malaysia.

<sup>2</sup>Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia.

<sup>3</sup>Department of Soil Science, Sindh Agriculture University Tandojam, Sindh 70060-Pakistan.

<sup>4</sup>Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400-UPM, Serdang, Selangor, Malaysia.

<sup>5</sup>Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia.

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The amounts of polyphenols at several stages of oil palm empty-fruit bunch (EFB) composting, the types of phenol compounds in several types of EFB composting processes and the effects of phenol compounds on EFB composting in an earthworm population were evaluated under field conditions. The amount of extractable phenols from decomposed EFB increased at the early stage of decomposition and decreased with increasing age of the EFB compost. The phenol content in soil with added EFB did not differ from that in the control soil. Under an open system and in the presence of soil, the phenol released from EFB easily degraded. The empty fruit bunches released phenol into their surroundings and no harmful effects were found on the earthworm population under the natural system. Using gas chromatograph-mass spectrometry (GC-MS), 2,4-bis(1,1-dimethylethyl) was identified in fresh EFB which was similar to the type of phenol compound in composted and field-decomposed EFB; 2,6-bis(1,1-dimethylethyl). In contrast, no phenolic compounds were detected in the vermicomposted EFB. This means that the vermicomposting process can be used to degrade toxic compounds such as phenol. Compared to normal compost, vermicompost contains fewer toxic compounds, which might be related to its advanced decomposition stages.

**Key words:** Earthworm population, oil palm waste, EFB composting, phenolic compounds.

## INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is considered a major driver of economic growth in developing countries, and as source of alternative fuel (biofuel). It is cultivated in about 43 countries in the world with an eight-fold growth in the

past 4 decades to over 12 million ha in 2009, while the area under cultivation in Malaysia increased by 5 times over the same period (Sheil et al., 2009; Teoh, 2010). It is estimated that by the year 2020 the world's production of oils and fats will increase to 174 M tonnes and the production of palm oil will reach 35 M tonnes (Ng, 2002). Therefore, palm oil will be the dominant vegetable oil throughout the world (Rajanaidu et al., 2000). The African oil palm is the most productive oil-bearing species (Basiron and Yew, 2009). One hectare of oil palm in good growing conditions produces about 4.5 t oil<sup>-1</sup> year<sup>-1</sup> and 0.50 t of palm kernel cake (PKC).

\*Corresponding author. E-mail: mmhanafi@agri.upm.edu.my.  
Tel: +603-89467842. Fax: +60389468968.

**Abbreviations:** EFB, Empty-fruit bunch; GC-MS, gas chromatograph-mass spectrometry.

Oil palm plantations produce two types of oil palm by-products: empty fruit bunches (EFB) and oil palm fronds. These by-products are produced in oil palm factories at the beginning of the oil extraction process and during oil palm frond pruning in plantations, respectively. About 6-7 tonnes of EFB are generated for every 10 tonnes of fresh fruit bunches (FFB) of oil palm. Annually, an average of 24 fronds is pruned per plant per year, and this is equivalent to 11.7 tonnes ha<sup>-1</sup> year<sup>-1</sup> (Chan, 2000). The rapid increase in the size of oil palm plantation areas and the huge production of oil palm waste has raised concerns over its disposal regarding the ecosystem structure and function.

Our previous studies (Sabrina et al., 2009) showed that *Pontoscolex corethrurus* and *Amyntas rodoricensis* died immediately when added into EFB as vermicultures. The number of *P. corethrurus* earthworms found in several oil palm plantations was low (0 to 42 individuals m<sup>-2</sup>); furthermore, no native earthworms were discovered. The disappearance of native earthworms and the colonization of exotic endogeics were reported in Peruvian Amazonia (Fragoso et al., 1999) and Brazilian Amazonia (Decaëns et al., 1999) when the forest was converted into agricultural land (Tsukamoto and Sabang, 2005).

A shift in land use might cause changes in microclimate conditions, especially moisture and food stock types for earthworms. A previous survey showed that food quantity, which indicates the organic carbon content was the principal component affecting earthworm populations in oil palm plantations. The oil palm by-products of fronds and EFB have frequently been added to soil as organic carbon sources. Oil palm fruit bunches contain lignocelluloses and cellulose (44.2%), hemicellulose (33.5%), and lignin (20.4%) (Azis et al., 2002). Phenol and its components are tremendously toxic and can easily be isolated from different sources; therefore, the occurrence of these compounds in the environment could cause environmental pollution (Kafilzadeh et al., 2010; Krishna et al., 2011). Phenolic compounds are found in lignin components and the degradation products of lignin can leach down from plant foliage/litter into the ecosystem; they have a high potential for environmental pollution (Wang et al., 2002). Out of ten (4-nitrophenol, n-nitrosodiphenylamine, 2,4,6-trichlorophenol, phenol, carbaryl, nitrobenzene, 1,2,4-trichlorobenzene, 1,2-dichloropropane, fluorine and dimethyl phthalate) of the most frequently found organic chemicals, phenols were found to be the most toxic chemical to earthworms (Neuhauser et al., 1986). Some phenolic compounds, such as pentachlorophenol (PCP), are classed as toxic compounds. A number of synthetic chemicals act like hormones (example, oestrogen) and interact with the human hormonal system: phenols are endocrine disruptors. Phenols also have effects on aquatic organisms, including bioaccumulation in the tissues of fish. Phenolic compounds are released from pesticides or preservative-treated waste wood, and some are also naturally present in plants. In addition, phenolic contents in lignin have a role

in nutrient availability in the soil.

The phenolic lignin residues in soil were shown to be covalently bound with N in the humic acid fraction (Schmidt-Rohr et al., 2004). The resulting chemical stabilization contributed to a long-term decrease in the availability of soil N and was associated with a decline in rice yields (Olk et al., 2006).

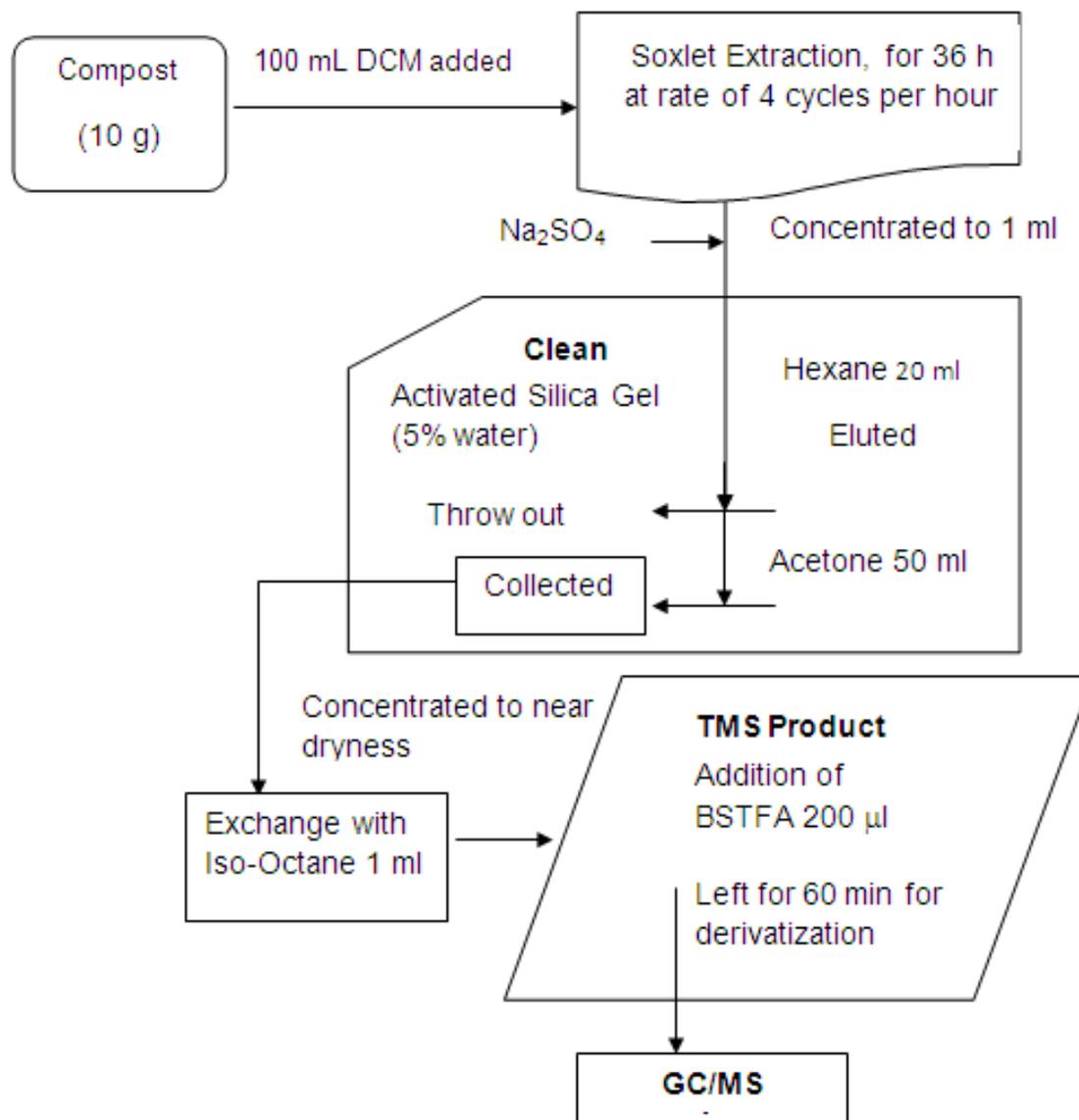
Earthworms offer cheaper way out to numerous social, financial and environmental problems plaguing the human society. Earthworms are key chemical degrader and a biological stimulator and degrade waste by several actions. They have the capability to bio-accumulate elevated contents of harmful chemicals including phenols in their tissues and either biodegrade or bio-transform them to nontoxic products with the aid of enzymes (Sinha et al., 2010). Earthworms have also been reported to bio-accumulate endocrine disrupting chemicals' (EDCs) from sewage. Significantly high concentrations of EDCs (dibutylphthalate, dioctylphthalate, bisphenol-A and 17  $\beta$ -estradiol) in tissues of earthworms (*Eisenia fetida*) are living in sewage percolating filter beds and also in garden soil (Markman et al., 2007). There is a general assumption that earthworms influence phenolic compounds in soils mostly by controlling microbial activity and changing their association with the mineral soil matrix. Earthworms strappingly enhanced microbial activity and thus mineralization of phenolic compounds in soils low in organic matter, clay content and microbial biomass compared to soils rich in these properties. The extra availability of clay in combination with earthworms reduced the mineralization of phenolic compounds in soil, suggesting that the intimate mixing during the gut passage through earthworms encourage the stabilization of phenolic compounds in casts (Butenschoena et al., 2009). Utilization of earthworms, in particular in the presence of plants, may be an ecologically sound and economically feasible technology to obtain a non-toxic, high-value product useful for agricultural purposes (Masciandaro et al., 2010).

Composting is one of the technologies that aim to use agricultural waste products and produce fertilizers from such wastes. Composting harmful pollutants in the field could minimize their negative effects on the surrounding environment, including earthworm populations; hence, the aim of this study was to determine the effect of fresh EFB applications on an earthworm population under natural conditions, the effect of the composting process on phenol concentrations, and the types of phenols present under several EFB conditions.

## MATERIALS AND METHODS

### Total extractable polyphenolic contents in decomposed EFB

The air-dried EFB was ground and passed through a 2 mm sieve. Samples were collected weekly during decomposition of the EFB. The samples were dried for 24 h in an oven at 60°C then ground, homogenized and sieved. Compost (10.0 g) was extracted using



**Figure 1.** Flow chart for the complete extraction and cleanup process of identifying phenolic compounds from compost sample.

100.0 ml methanol over 24 h at a rate of four cycles  $\text{h}^{-1}$  in a Soxhlet extraction apparatus. Then, the extracts were colorimetrically assayed by the Folin-Denis method, using gallic acid as the calibration standard. The procedure consisted of diluting an aliquot of the extract solution (1.0 ml) in water (20.0 ml), followed by the addition of the Folin-Dennis reagent (0.5 ml). After 3 min, 10.0 ml of an aqueous  $\text{Na}_2\text{CO}_3$  solution ( $17.0 \text{ g } 100 \text{ ml}^{-1}$ ) was added, mixed in a vortex, and left to stand at room temperature for 1 h. Absorbance was measured after 1 h at 760 nm against a blank. The results were expressed as g GAE (gallic acid equivalents)  $100 \text{ g extract}^{-1}$  (Singleton et al., 1999).

#### Types of phenols in the decomposed EFB

Three types of decomposed EFB: (i) vermicompost EFB, (ii) normally composted EFB, and (iii) EFB naturally decomposed in the field, plus a control: fresh EFB. The samples were extracted using

100.0 ml dichloromethane (DCM) over 36 h at the rate of four cycles  $\text{hour}^{-1}$  in a Soxhlet extraction apparatus. Anhydrous  $\text{Na}_2\text{SO}_4$  was added to remove water. The extract was cleaned up using 20.0 ml hexane and collected by 50.0 ml acetone passing through a column filled with activated silica gel (Helaleh et al., 2001). Then, the extractant was concentrated into 1.0 ml using a rotary evaporator and was subsequently followed by nitrogen gas blow-down until dryness. The solvent was changed to 1.0 ml iso-octane (high-performance liquid chromatography (HPLC) grade, boiling point (B.P)  $98.9^\circ\text{C}$ ) (Figure 1). Afterwards, a derivatization step was carried out using 200  $\mu\text{l}$  *N,O*-bis(trimethyl-silyl) trifluoroacetamide (BSTFA) and leaving the mixture for 60 min for complete derivatization.

A 1.0  $\mu\text{l}$  aliquot of the extract was injected into a gas chromatograph using the splitless mode with the split vent closed for 5 min. The gas chromatograph-mass spectrometry (GC-MS) system used in this experiment was a GC-17A, gas chromatograph (Shimadzu, Kyoto, Japan) filled with GCMS model: QP5050A

**Table 1.** GC-MS conditions.

<b>Gas chromatography</b>	<b>Mass spectrometry</b>
Total flow 50.0 ml min <sup>-1</sup>	Interface temperature 270 °C
Head column pressure 10 Kpa (5 min), 70 k pa (25.47 min)+1.0 Kpa min <sup>-1</sup>	Electron multiplier voltage 1.20 kV
Injector temperature 300 °C	Scan mode ( <i>m/z</i> ) 35 – 450
Injected volume 1 µl	
Oven program 50 °C (5 min) + 8 °C min <sup>-1</sup> , 150 °C (0 min) + 7 °C min <sup>-1</sup> , 200 °C (9.58 min) + 5 °C min <sup>-1</sup> , 250 °C (0 min) + 8 °C min <sup>-1</sup> , 300 °C (10 min)	

(benchtop quadrupole mass spectrometer). Class-5000 software (Shimadzu, Kyoto, Japan) was used for data processing. A BPX 5, a chromatographic fused-silica capillary column (made by J and W Scientific, California, USA) was also used. The column dimensions were 30 m × 0.258 mm and the film thickness was 0.25 µm. The chemical nature of the liquid phase was methylpolysiloxane (35% phenyl). Helium gas was used as the carrier gas (purity 99.99%). The GS-MS parameters are given in Table 1.

#### Location of the experiment

The field experiment was conducted at share farm 10 of the Universiti Putra Malaysia (UPM) oil palm experimental field, Serdang, Malaysia.

#### Effect of EFB on the earthworm population under field conditions cage preparations

There were 3 cages in each replication for each treatment. The cages were prepared using a PVC cylinder (40 cm in length and 20 cm in diameter). The cylinder was buried down to 10 cm vertically, leaving 30 cm exposed at the soil surface. The bottom and top of the cylinder were covered with mosquito netting to prevent the earthworms escaping from the cage. About 10 kg of Bungor soil (type: Kandiuult) was packed to the bulk density of Bungor soil (Figure 2).

#### Earthworms and EFB inoculation

Five earthworms were introduced into each cage before the treatments were added (Figure 2). The treatments were: control, 37.5 t ha<sup>-1</sup> raw EFB, and 37.5 t ha<sup>-1</sup> composted EFB, with three replicates. All residues (treatments) were added to the surface of the soil inside each cage. The cages were exposed to natural condition and water was applied to each cage to at least the field capacity level.

At the end of the experiment, the population of earthworms and the number of egg was counted, organic carbon was analysed using the method of Walkley and Black (1934) method, total nitrogen was determined by the Kjeldahl method (Bremner, 1960) and the total extractable phenol contents in the top soil (0 to 10 cm) of the cage were analysed using the modified method of Singleton and Rossi (1965).

## RESULTS AND DISCUSSION

### Total extractable phenols in composted EFB

The amount of phenol sharply increased in the first week,

attained a maximum in the third week, and gradually decreased thereafter (Figure 3). In contrast, the total amount of extractable phenol released from the EFB applied to the Bungor soil in the open incubation system gradually decreased with time. The trend of total extractable phenol in the present study was similar to the trend of percentage lignin remaining in the EFB obtained in a previous study (Zaharah and Lim, 2000). Our findings indicate that phenol in the EFB was broken down during EFB decomposition. As the incubation occurred in a closed system, the phenols accumulated during the first to third weeks. Due to the possibly low population of bacteria at that stage, phenol was slowly degraded. Under natural conditions phenol was degraded easily and leached out from the system. If phenol is the factor causing the death of earthworms during the vermin-composting process, pre-composting the EFB for up to 4 weeks might be very useful for decreasing the negative effects of phenol on earthworms.

Some types of phenol, such as chlorinated phenols, are slowly degraded and do not occur naturally in the environment. Due to resistance to microbiological degradation, chlorinated phenols persist for many years in the environment, leading to the accumulation of these poisonous molecules (Meunier and Sorokin, 1997). In general, the increased chlorination of organic compounds increases the recalcitrance or stability of the molecule in the environment. Hence, they are used as preservatives; for example, pentachlorophenol is used as an insecticide for termite control and as a preservative for wood, wood products, starches, dextrans, and glues. Phenol compounds in EFB are expected to contain tannins, chlorogenic and benzoic acid derivatives, and procyanidin oligomers, which are the phenol compounds mostly found in lignocellulosic residues (Pinelo et al., 2004). As a result of intensive insecticide spraying in oil palm fields, EFB might contain chlorinated phenols.

### The types of phenolic compounds in raw and decomposed EFB

Many peaks can be observed in the fresh EFB sample (Figure 4i), but only one peak belongs to a phenolic compound. This peak appears at a retention time of 15.967 min. The only phenolic compound found in fresh

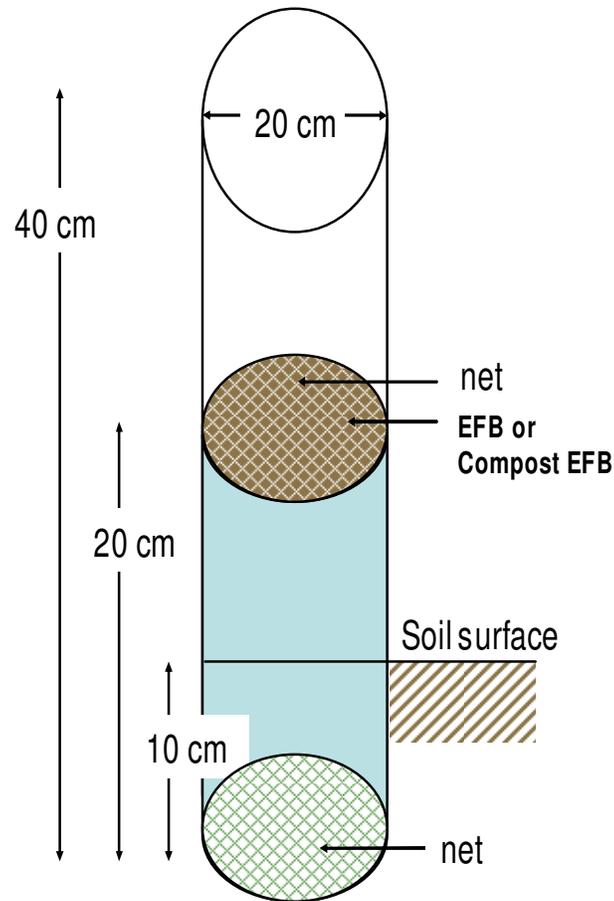


Figure 2. Earthworm caging used in the field experiment.

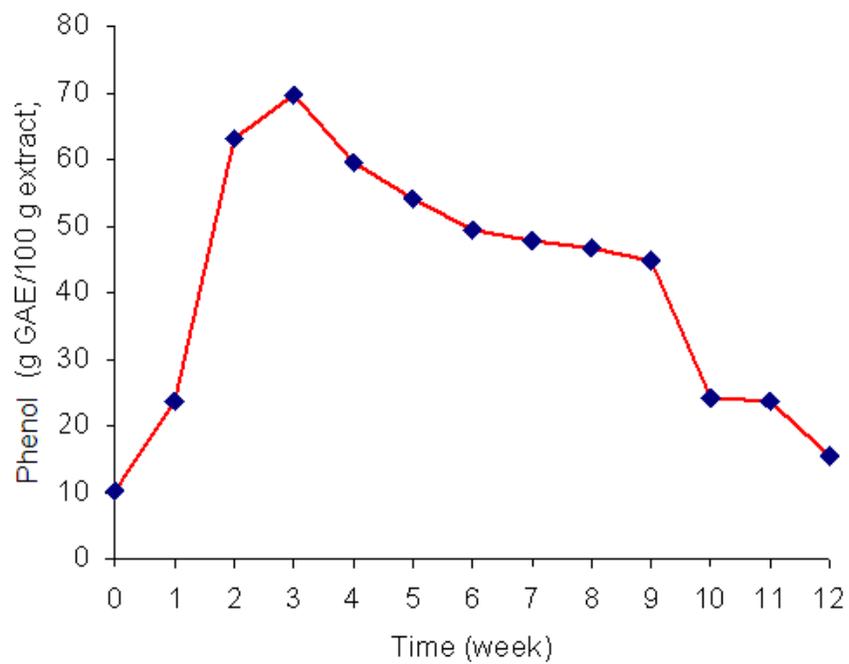
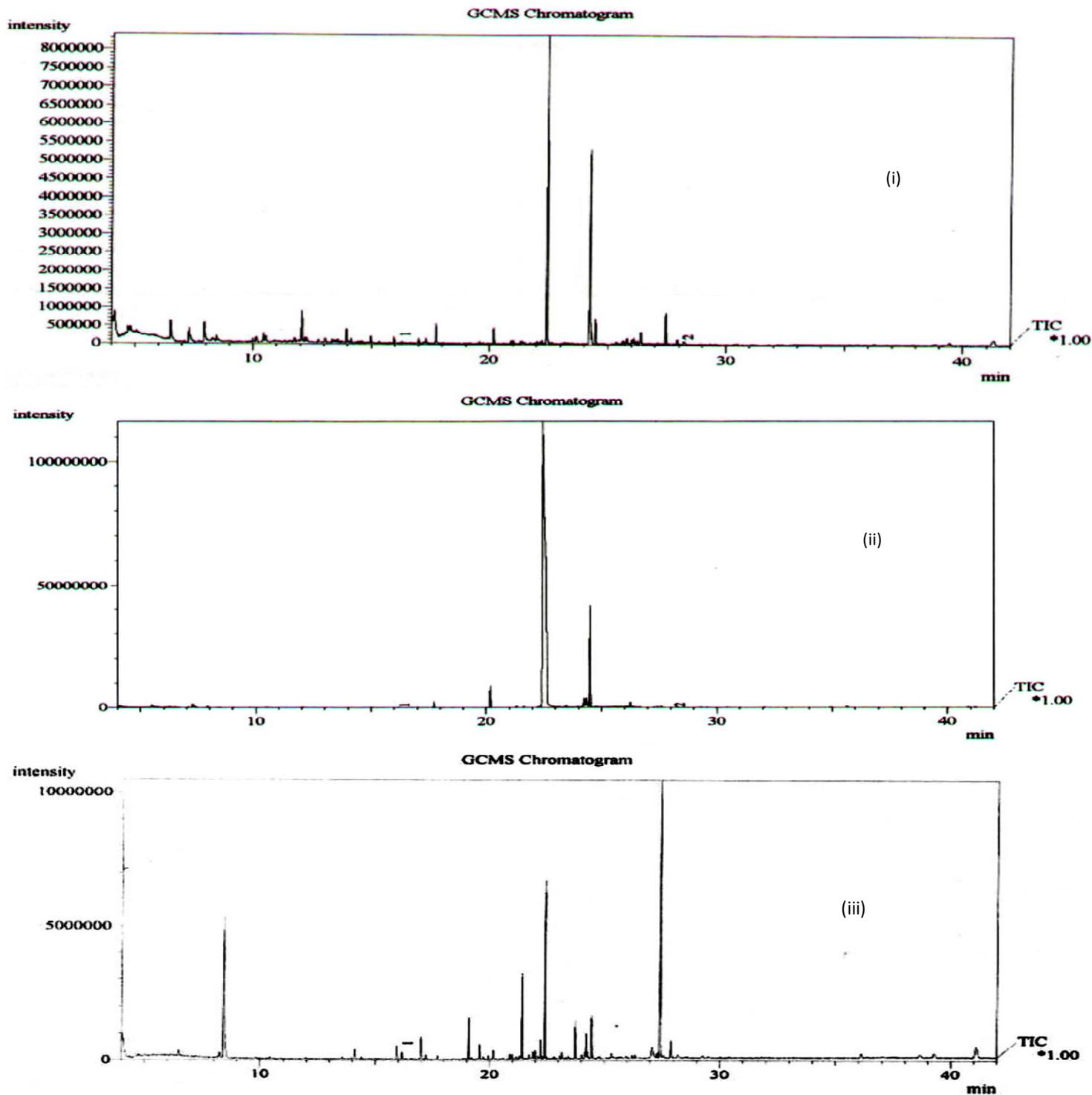


Figure 3. Total extractable phenol in decomposed EFB.



**Figure 4.** Chromatogram of the phenolic compounds (BSTFA derivatives) of (i) fresh EFB, (ii) field composted EFB, (iii) composted EFB and (iv) vermicompost.

EFB by gas chromatography-mass spectrometry (GC-MS) was 2,4-bis(1,1-dimethylethyl) phenol. The vermicompost did not contain any phenols, whereas the conventionally composted EFB also contained 2,4-

bis(1,1-dimethylethyl) phenol, appearing at a retention time of 15.950 min (Figure 4). The EFB naturally composted in the field contained 2, 6-bis (1,1-dimethylethyl) phenol, which appeared at a retention time of 19.958 min.

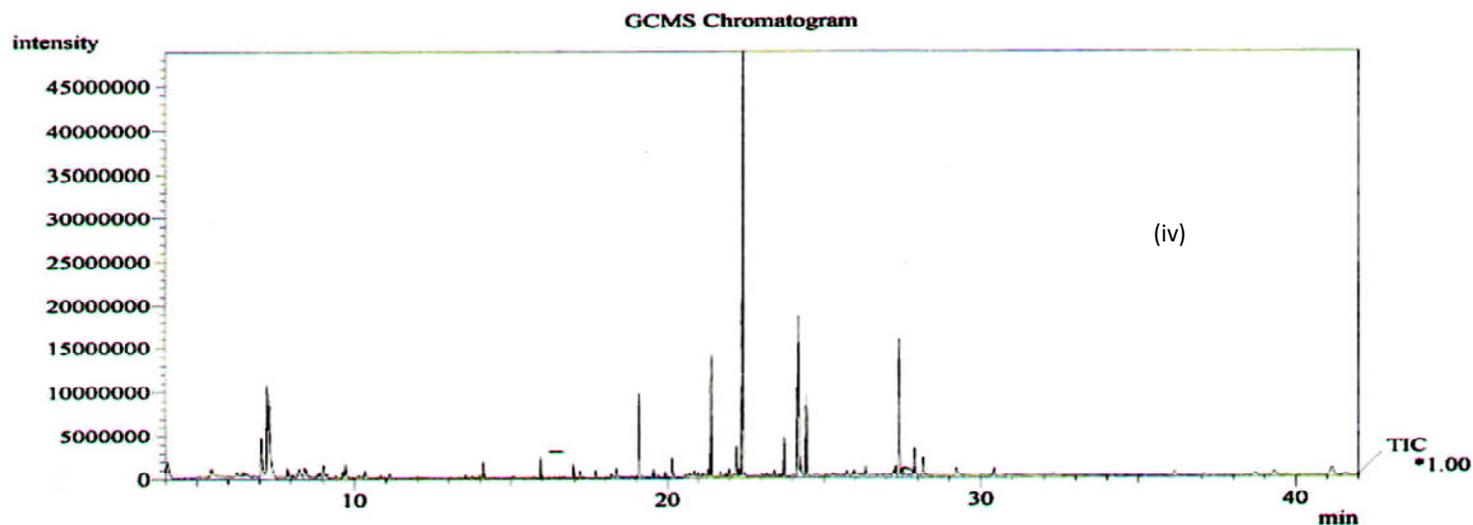
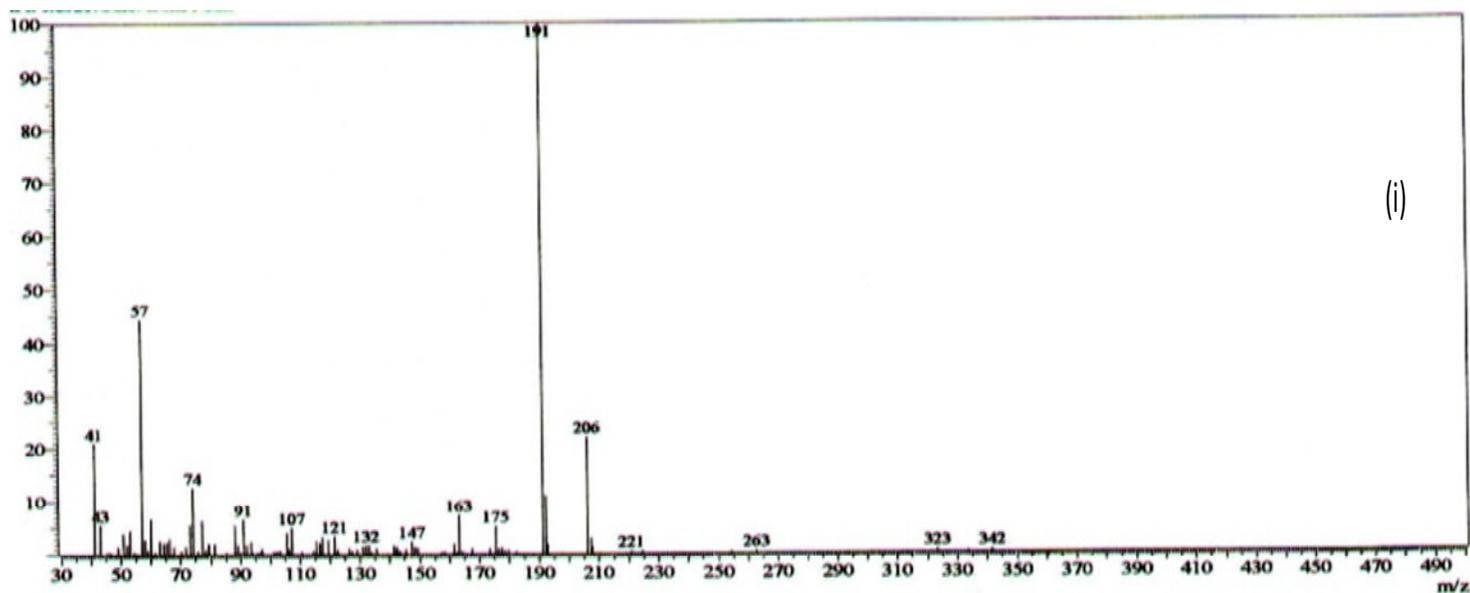


Figure 4. Contd.



**Figure 5.** Mass spectrum of (i) 2,4-bis(1,1-dimethylethyl)phenol of fresh EFB, (ii) 2,6-bis(1,1-dimethylethyl)phenol of field composted EFB, (iii) 2,6-bis(1,1-dimethylethyl)phenol of composted EFB and (iv) vermicompost EFB (BSTFA derivative).

The 2,4-bis(1,1-dimethylethyl) phenol had a molecular ion at 206 m/z, and the most intense peak at m/z 191 (Figure 5). Phenols are electron-rich, polar, acidic, aromatic compounds. For the fragmentation pattern (Figure 6), the initial molecule was observed at m/z 206,  $C_{14}H_{22}O$ . This peak was very low, meaning that this compound is very fragile. Methyl groups were attached to the phenol ring in the positions *ortho* and *para*. The first fragmentation molecule with a molecular weight of 206 discharges the methyl group ( $-CH_3$ ) to give a peak at 91 m/z. The peak at 191 is the highest peak and is called as

“baseline”. The next fragmentation step releases  $H_2C=CH_2$ , breaking the structure and resulting in a molecular weight 163. Phenols containing longer hydrocarbon chains undergo benzylic cleavage of the chains, resulting in hydroxyl tropyllium ions at m/z 91. Firstly, the phenol fragments observed at m/z 74 were simple benzene derivatives, with a loss of a methyl radical ( $CH_3CH_2CH_2CH^+$ ) at m/z 57 (Figure 6). The structures of the phenol compounds in fresh, field-composted and composted EFB are shown in Figure 7. The 2,6-bis(1,1-dimethylethyl) phenol and the 2,4-bis(1,1-dimethylethyl)

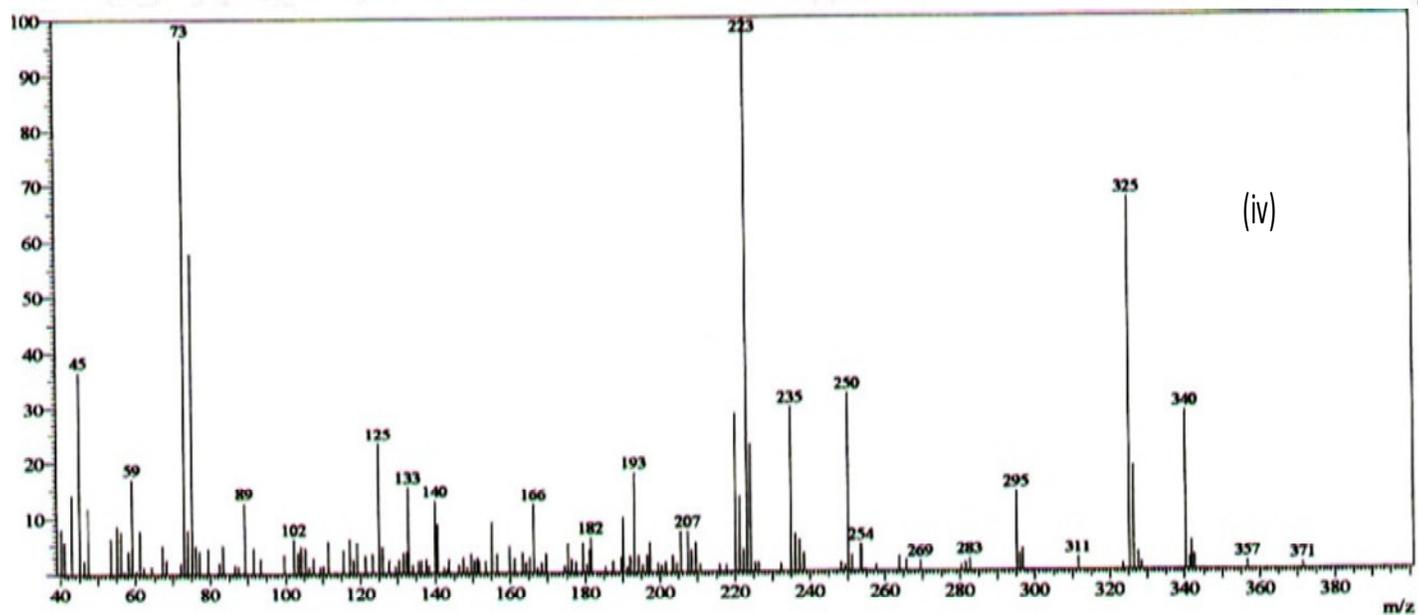
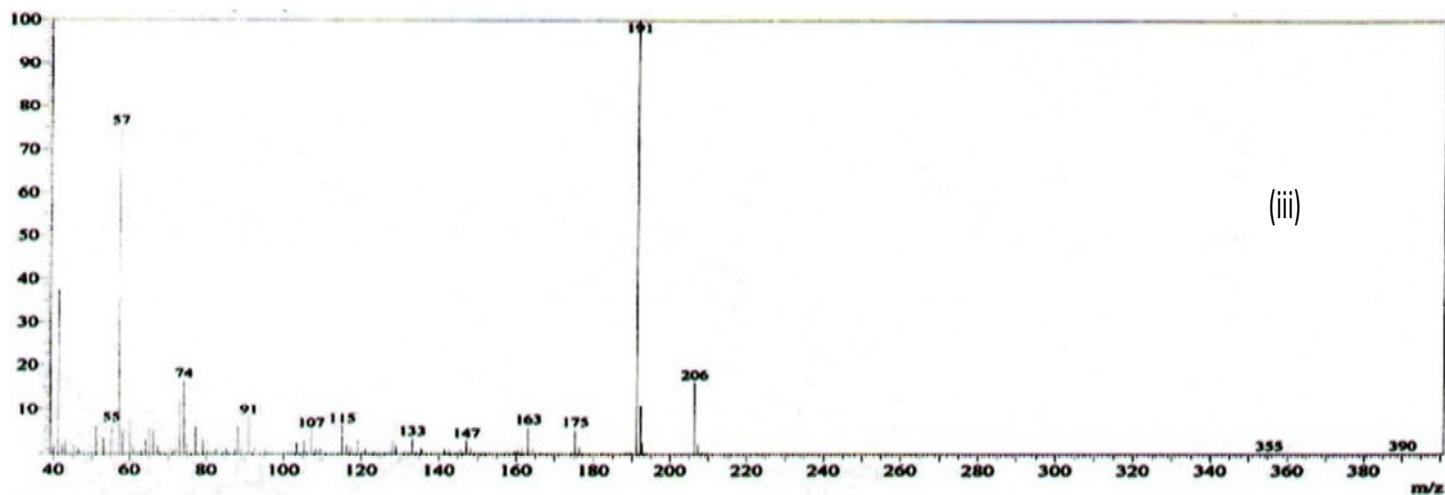
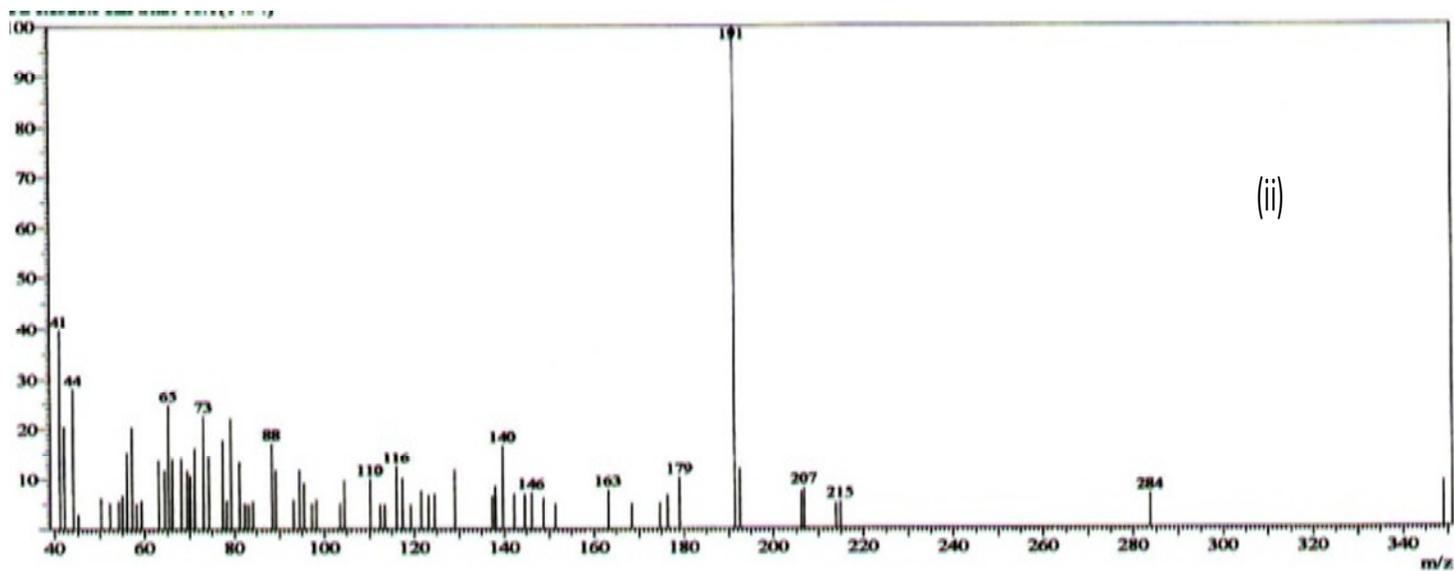


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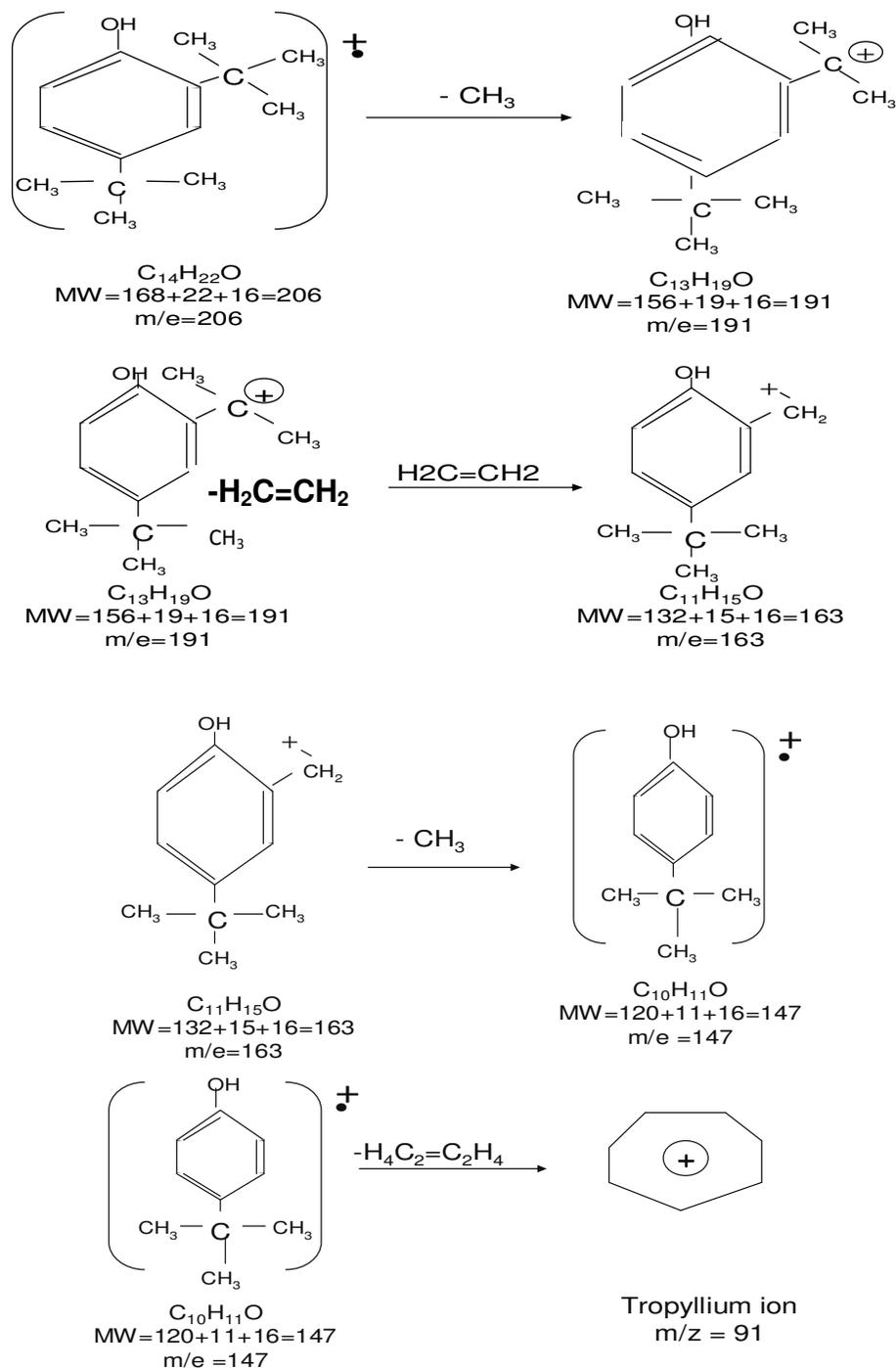


Figure 6. Fragmentation of phenol.

phenol were isomers.

### Effect of EFB on the earthworm population

The number of earthworms decreased from five individuals to four for the composted EFB, three individuals for the fresh EFB, and one for the control by the end of this

study (Table 2). However, no fecundity was observed during this experiment for any of the earthworms in any of the treatments. Even though the number of earthworms was very low for the treatment without organic carbon (control), there was no significant relationship between the earthworm population and organic carbon content. The relationships between the earthworms and total N in the soil and between the earthworm population and the

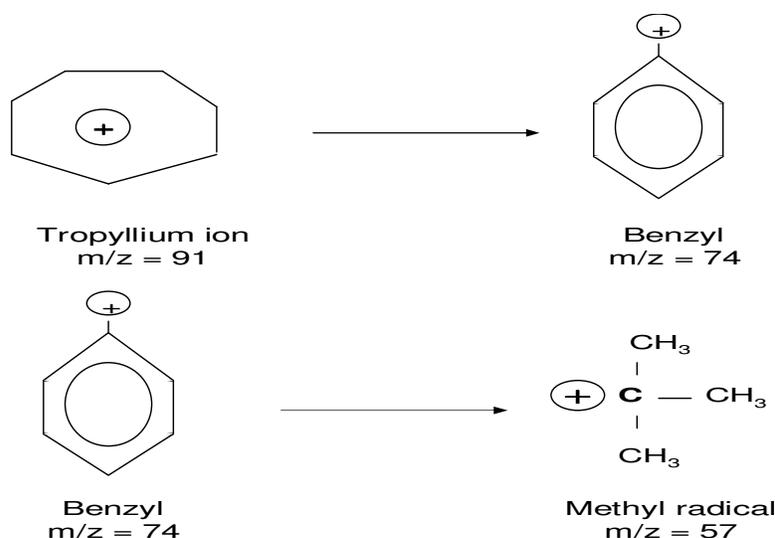
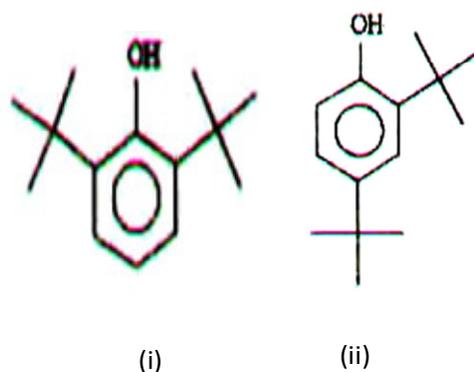


Figure 6. Contd.



**Figure 7.** Structure of (i) 2,6-bis(1,1-dimethylethyl)phenol found in field composted and composted EFB, and (ii) 2,4-bis(1,1-dimethylethyl)phenol of fresh EFB.

total extractable phenol inside the cage were also non-significant. The raw EFB contained 52% organic C, while the composted EFB contained 30% organic C. The concentration of total extractable phenol in the composted EFB after 3 months decreased to a very low level, similar to that of the control treatment, and no significant relationship was found between the total extractable phenol and the earthworm population. This result suggests that the EFB was not a factor in reducing the population of earthworms and was thus safe to be added to the soil. The reason why *P. corethrus* and *A. rodericensis* died in the EFB media might have been due to the absence of soil during the vermicomposting process. The earthworm *P. corethrus* is an endogeic species, whereas *A. rodericensis* is an anecic species (Edwards and Bohlen, 1996). Both of those species live

in soil and are not suitable for vermicomposting processes. In Peru and India, *P. corethrus* was reared in order to produce casts for improving the physical structure and productivity of the soil (Senapati et al., 1999).

## Conclusion

In the closed system, the amount of extractable phenol from decomposed EFB increased in the early stages of decomposition and then gradually decreased. However, under field conditions or in an open system, 3 months after the EFB application the amount of extractable phenol in soil with EFB was low and equal to the phenol content in the control soil. The application of fresh EFB

**Table 2.** The effect of application of empty fruit bunch (EFB) on earthworm population, organic carbon, nitrogen and total extractable phenol.

Treatment	Earthworm	Organic C (%)	Total N (%)	Total Phenol (g GAE g <sup>-1</sup> extractant)
Control	1 b	10.63 b	0.61 a	3.6 a
EFB fresh	3 a	12.19 a	0.66 a	4.2 a
Composted EFB	4 a	11.54 ab	1.03 a	3.1 a

Means with the same letter in each column are not significantly different at P=0.05 level. GAE, Gallic acid equivalent.

under natural conditions did not drastically reduce the earthworm population. Fresh, composted, and field-composted EFB produced phenol compounds, whereas no phenolic compounds were detected in vermicomposted EFB.

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