



Evaluation of Composting with ‘Spent’ Mushroom Substrate and Sawdust for Remediation of Petroleum Hydrocarbon-Polluted Soil

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Authors' contributions

This work was carried out in collaboration between both authors. Author EA designed the study, wrote the protocol, anchored the field study, gathered and interpreted the data and produced the initial draft. Author DIA managed the literature searches and performed data analysis. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the capacities of composting ‘spent’ mushroom substrate and sawdust with crude oil polluted soil to enhance degradation of petroleum hydrocarbon.

Study Design: The degradation of petroleum hydrocarbon contents of crude oil polluted treatments composted with ‘spent’ mushroom substrate, sawdust and a mixture of ‘spent’ mushroom substrate and sawdust were compared with that of crude oil polluted-not-composted treatment to determine the influence of the compost amendments.

Place and Duration of Study: The Centre for Ecological Studies, Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria, from March - July 2015.

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Methodology: Five buckets were each filled with 1500 g of top alluvial soil and labelled T₁ – T₅. T₁ (i.e. control) was not polluted while the other buckets were each polluted with Bonny Light crude oil at 6.7% (v/w). T₂ was not composted with any material after pollution. T₃ was mixed with 200 g of 'spent' mushroom substrate and T₄ with 200 g of sawdust while T₅ was composted with a mixture of 200 g of 'spent' mushroom substrate and 200 g of sawdust.

Results: Final pH of 8.4±0.1 and 6.4±0.2 were recorded in T₁ and T₅ respectively. The pH of T₂ decreased from 8.8±0.1 to 7.1±0.1 and from 8.7±0.1 to 7.1±0.4 in T₃, but increased from 5.3±0.0 to 8.5±0.0 in T₄. Percentage total petroleum hydrocarbon contents decreased by 75.5% in T₃ and T₅ and 64.9% in T₄. Average phosphorus contents increased from 0.33±0.0 to 52.60±0.23 mg/kg in T₅, 0.33±0.03 to 1.81±0.35 mg/kg in T₄ and from 0.36±0.01 to 1.34±0.62 mg/kg in T₃ but decreased from 0.35±0.01 to 0.14±0.60 mg/kg in T₂. Total nitrogen contents recorded reduction in T₂, T₃, T₄ and T₅ and total organic carbon increased in T₂, decreased in T₄ and was constant in T₅ during the experiment.

Conclusion: 'Spent' mushroom substrate, sawdust and a mixture of 'spent' mushroom substrate and sawdust composted with crude oil polluted soil significantly enhanced removal of soil hydrocarbon content.

Keywords: Pollution; crude oil; bioremediation; composting; 'spent' mushroom substrate; sawdust.

1. INTRODUCTION

Soil as a natural resource is important to man. Humans depend on it for food production and to sustain rangeland and forest. However, the activities of man have adversely affected the environment, soil inclusive. The operations of the oil and gas industry in Niger Delta of Nigeria has led to widespread petroleum hydrocarbon environmental pollution in the area. Petroleum hydrocarbon spills occur through different means, some of which have been reported by Achebe, et al. [1-3].

Petroleum hydrocarbon environmental pollution lead to the destruction of arable lands and shorelines used for crop cultivation in crude oil-producing localities in Niger Delta. Furthermore, hydrocarbon soil pollution has been reported to lead to a reduction in the size of farmlands and decline in agricultural production in the area [4].

Several bioremediation techniques are used to clean up oil-polluted terrestrial habitats. Composting, a low-tech and cost-effective bioremediation technique, was tested using 'spent' mushroom substrate and sawdust. 'Spent' mushroom substrate was reported to be rich in mineral nutrients [5,6] and sawdust, was reported to improve soil hydro-physical properties [7-9]. Based on these properties of these materials, this work investigated composting crude oil-polluted soil with 'spent' mushroom substrate, sawdust and a mixture of both to enhance the degradation of the hydrocarbon content of crude oil polluted soil. The test materials used in this research are locally available and composting is

a simple technology that does not require advance skill for its implementation. Therefore, remediation of hydrocarbon polluted soil by composting with 'spent' mushroom substrate and sawdust or the mixture of both can be used to clean-up small to medium scale hydrocarbon pollution in terrestrial habitat Niger Delta.

2. MATERIALS AND METHODS

This research was carried out at the Centre for Ecological Studies which is located at latitude 4° 54' N and longitude 6° 55' E in the University of Port Harcourt, Nigeria. Top alluvial soil with no previous petroleum hydrocarbon pollution was collected from a fallow land within the University premises. The soil was air-dried and sieved through 2-mm soil mesh. One thousand five-hundred gram (1500 g) each, of the processed soil weighed with a digital weighing balance (model: Ohaus Scout II SC4010) was transferred into five (5) buckets (dimensions: 20 cm height by 18 cm diameter; 5000 cm³ volume) with bottom perforations to enable it drain off water from the soil placed in it. The soil was immediately wetted with 500 ml of distilled water to simulate natural condition. The completely randomized design was used to assign buckets to treatments and control and labeled T₁, T₂, T₃, T₄ and T₅. T₁ was set as the control and was therefore not polluted while T₂, T₃, T₄ and T₅ were each polluted with Bonny Light (i.e. crude oil) at 6.7% (v/w) by soil surface application. The buckets were after pollution allowed for 24 hours for the oil to infiltrate into the polluted soil. T₂ was not integrated with any material after pollution while T₃, T₄ and T₅ were mixed 200 g of 'spent'

mushroom substrate, 200 g of sawdust and a mixture of 200 g each of 'spent' mushroom substrate and sawdust respectively. The composted treatments and the control were replicated five (5) times, shaded from sunlight and watered with 500 ml of distilled water twice weekly.

Soil samples were collected from each set up 24 hours after pollution (24 HAP) for soil chemical properties analyses before the composted treatments were mixed with the different materials. Further samplings were carried out on the 30th and 150th days after pollution. Soil pH was determined by meter method with a pH meter (Hanna HI 8314) from a slurry of soil sample in distilled water at the ratio of 1:1 (w/v). Total petroleum hydrocarbon (TPH) was determined by gas chromatography with gas chromatograph (HP 5890 model) equipped with flame ionization detector (FID) using air-dried soil sample after extraction of its total petroleum hydrocarbon content in dichloromethane as the extraction solvent. Percentage TPH removal was calculated using the formula:

$$\% \text{ TPH Removal} = \frac{\text{Initial Treatment TPH} - \text{Final Treatment TPH}}{\text{Initial Treatment TPH content}} \times 100$$

Total organic carbon (TOC) content was determined by titrimetric method (i.e. Walkley - Black technique) while total organic matter was determined by calculation using the formula of Combs and Nathan [10]. Soil total nitrogen and average phosphorus contents were determined by spectrophotometry with a spectrophotometer (HACH DR/890 colorimeter).

Means and standard deviations of data collected were calculated. One-Way Analysis of Variance (ANOVA) using standard procedure and Fisher's Least Significant Different (LSD) t-test were carried out at $P = 0.05$. Results obtained were presented in line graphs and tables.

3. RESULTS AND DISCUSSION

3.1 pH

The effects of composting crude oil polluted soil with 'spent' mushroom substrate and sawdust on soil pH varied with treatment (Fig. 1). pH of T_1 was alkaline (i.e. 8.4 ± 0.1) and differed significantly with that of T_5 . This was expected because it was not polluted nor composted with any of the remedial materials. This observation differed with the findings of Osuji and Nwoye [11] who reported acid pH on crude oil polluted soil.

Initial pH of T_2 and T_3 were alkaline while those of T_4 and T_5 were acidic. The reason for this difference in initial pH characteristics was not known. Final pH of T_2 and T_3 were neutral (i.e. reduction in pH). The change in pH observed in T_2 was attributed to the crude oil treatment. This was explained by Essien and John [12] to be the effect of organic acids which was produced from microbial degradation of the crude oil. The pH reduction in T_3 agreed with the findings of Danai et al. [13] and was attributed to the 'spent' mushroom substrate composted in the treatment. This could have resulted based on the explanation of Essien and John [12] or, could have resulted because of the influence of the pH characteristics of the 'spent' mushroom substrate composted in the treatment. The pH of T_4 increased from 5.3 ± 0.0 to 8.5 ± 0.0 (i.e. turned alkaline). This suggested that the sawdust mixed with the treatment was responsible for the change in pH. Sources of organic matter such as sawdust are known to act as a buffer, therefore it may have played such a role in the treatment which resulted in the increase in pH of the treatment from acid to alkaline. This can be justified by the fact that the treatment was only composted with sawdust. There was no change in pH in T_5 . This was speculated to be the result of the counteractive interaction of the mixture of 'spent' mushroom substrate and sawdust of the treatment. We therefore propose that composting crude oil-polluted soil with 'spent' mushroom substrate reduced pH while sawdust increased pH. Statistically, there were no significant differences between mean pH of treatments at $P = 0.05$.

3.2 Total Petroleum Hydrocarbon Content

Initial and final total petroleum hydrocarbon (TPH) contents of T_2 , T_3 , T_4 and T_5 were high compared with that of T_1 (Fig. 2). The TPH of T_1 was below the maximum acceptable limit (i.e. 50 mg/kg) prescribed by the Environmental Guidelines and Standards for the Petroleum Industry in Nigeria, EGASPIN [14] for determination of hydrocarbon pollution. Low TPH in T_1 was in line with [11] and was expected because it was not polluted. There were reductions in TPH contents in T_2 , T_3 , T_4 and T_5 . The reduction in T_2 was lost through natural attenuation. The decline in TPH in T_3 was in line with Eramo [15] who studied bioremediation of diesel-contaminated soil with 'spent' mushroom compost. The TPH reduction recorded in this study was attributed to result because of the 'spent' mushroom substrate composted in the

treatment. Okerentugba et al. [16] stated that 'spent' mushroom compost is never sterile but rich in consortium of hydrocarbon-degrading microorganisms such as fungi. Therefore, the high TPH reduction may have resulted from biodegradation activities of the fungal organisms in the substrate. This explanation is supported by Adenipekun and Isikhuemhen [17]; Okwujiako et al. [18] who in separate studies tested *Lentinus squarrosulus* and *Pleurotus florida* mycelia for bioremediation of engine oil-polluted soil. According to Okwujiako et al. [18], fungal mycelia biodegrade organic compounds with the aid of their extracellular enzymes and acids. Another plausible explanation was based on the report of Fidanza et al. [5] which stated that 'spent' mushroom substrate is rich in mineral nutrients. Therefore, increase in nutrient status through released from the substrate may have enhanced the metabolic activities of indigenous hydrocarbon-degraders in the composted medium to degrade TPH. The TPH reduction in T₄ was the result of the sawdust composted in the treatment and this agreed with Bodoosingh et al. [19], Albert and Anyanwu [20]. According to Felsot and Dzantor [21], this may have resulted because sawdust serves as an alternative source

of carbon for hydrocarbon-degrading microbes. This may have enhanced the metabolism of hydrocarbon-degraders which consequently resulted in the observed high TPH degradation of the treatment. The Reduction in TPH of T₅ was acceptable because each of 'spent' mushroom substrate and sawdust composted alone with crude oil-polluted soil enhanced TPH degradation. Therefore the result of their combination as seen in the treatment was acceptable. Finally, 'spent' mushroom substrate and sawdust composted with crude oil polluted soil stimulated TPH degradation.

3.3 Percentage TPH Reduction

Percentage TPH reduction varied with the treatment applied (Table 1). Initial and final TPH of T₁ were negligible because it was not polluted. However, TPH reduced by 30% in T₂. At the end of the study, percentage TPH reduction was higher in T₃, T₄ and T₅ compared with T₂. This was because they were composted with the respective materials under evaluation in this research. Therefore, 'spent' mushroom substrate, sawdust and their mixture enhanced TPH reduction.

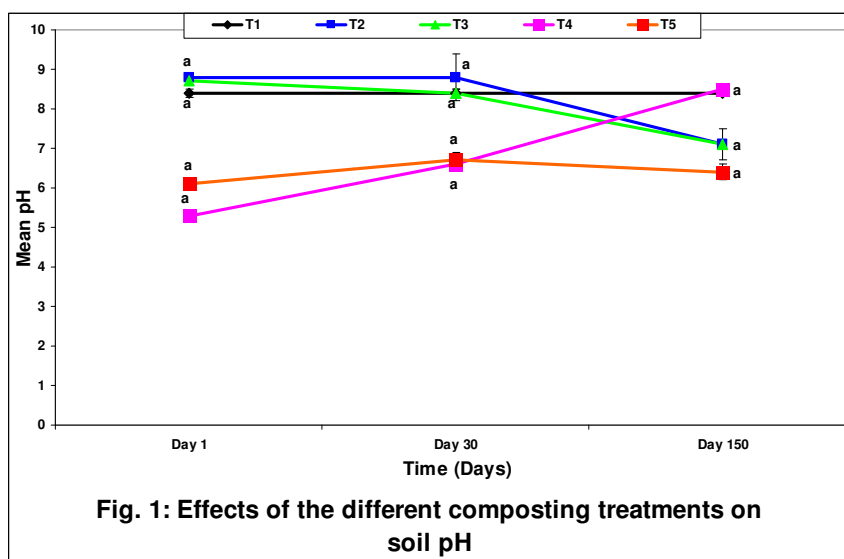


Table 1. Percentage TPH reduction in treatments

Treatment	Mean initial TPH content (mg/kg)	Mean final TPH content (mg/kg)	Percentage TPH reduction (%)
T ₁	0.92±0.78	0.44±0.33	Negligible
T ₂	1796.21±200.89	1249.97±108.95	30.41%
T ₃	2495.30±526.70	611.30±72.63	75.50%
T ₄	2485.37±83.07	871.03±69.84	64.95%
T ₅	2590.20±91.30	642.25±143.33	75.20%

3.4 Average Phosphorus

Average phosphorus (AP) was low in T₁ but high and changed in amount with time in T₂, T₃, T₄ and T₅ (Fig. 3). The low average phosphorus of T₁ was acceptable because it was not composted with any of the materials under study. Decrease in AP of T₂ is in line with Bello and Anobeme [22] who evaluated the effects of petroleum hydrocarbon spills on soil chemical properties. The reduction in AP of T₂ implicated crude oil to be responsible for the observation. The plausible explanation for this observation might be that the

pollutant immobilized soil phosphorus. Average phosphorus content in T₃ showed a direct relationship with time. This observation implied that 'spent' mushroom substrate was the source of input of the nutrient. This agreed with Orluchukwu and Adedokun [23] who also reported increase soil phosphorus in 'spent' mushroom substrate-amended soil. Final average phosphorus increased significantly in T₄ over its initial concentration and this suggested that the sawdust was the source of input of the nutrient. This observation was in agreement with Awodun, et al. [24]. A similar increase in soil

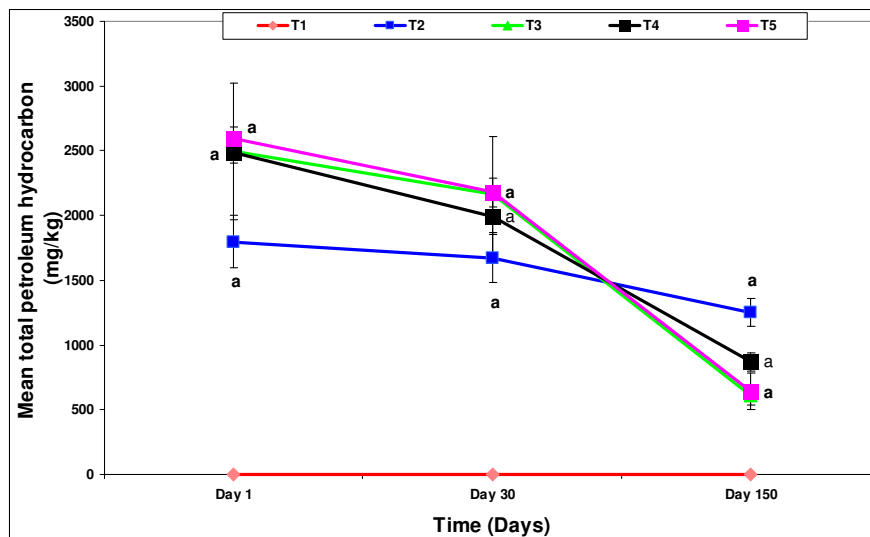


Fig. 2: Effects of the different composting treatments on soil total petroleum hydrocarbon

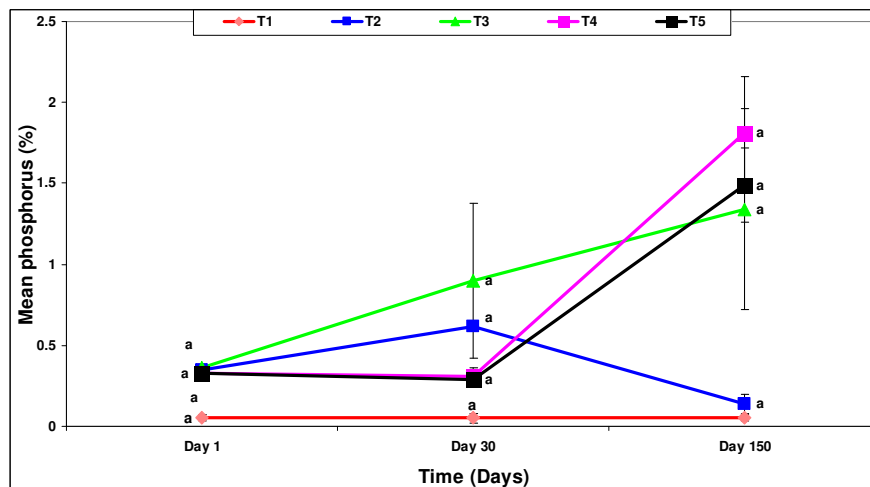


Fig. 3: Effects of the different composting treatments on soil phosphorus

average phosphorus content was recorded in T₅. From the results obtained, the test materials enhanced average phosphorus contents but there were no significant statistical differences between treatments at $P = 0.05$.

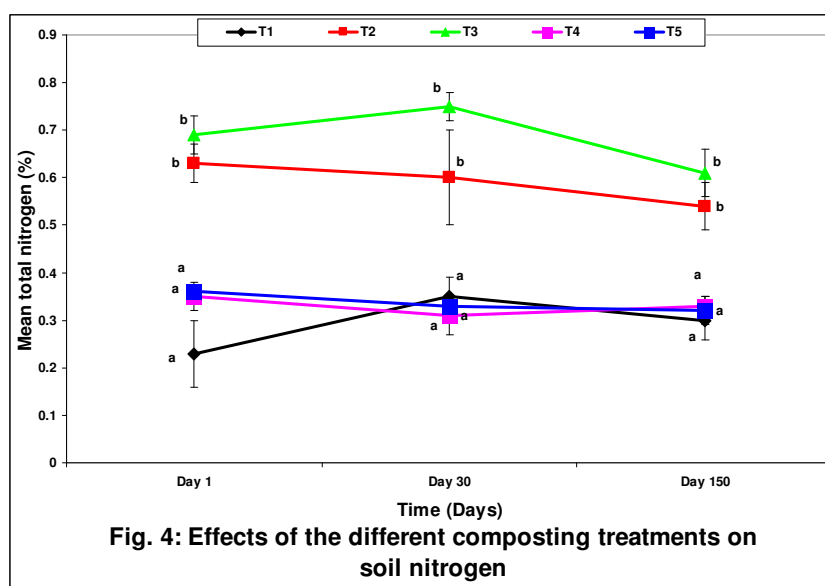
3.5 Total Nitrogen

Total nitrogen, TN was reduced in T₂, T₃, T₄ and T₅ but increased in T₁ with time (Fig. 4). TN result of T₁ differed significantly compared with T₂ and T₃. The observation on T₁ was acceptable, and the decrease in T₂ was in line with [11,22]. This hinted that crude oil spill reduced soil total nitrogen content. A similar reduction of the nutrient in T₃ was reported by Roy, et al. [25] from the evaluation of 'spent' mushroom substrate soil amendment as biofertilizer for growth improvement in *Capsicum annum*. It was explained by Roy et al. [25] to result from immobilization of nitrogen by the pollutant and this reduction was not improved by composting with 'spent' mushroom substrate. Total nitrogen in T₄ was reduced on the 30th day and increased on the 150th day. The initial reduction could have been the result of immobilization of the nutrient by the pollutant as was observed in T₂. However, the increase in total nitrogen content on the 150th day could have resulted from the release of the nutrient from the decomposition of the composted organic material and release of the earlier immobilized molecules of the nutrient from nitrogenous compounds. The mixture of 'spent' mushroom substrate and sawdust (i.e. T₅) recorded slight reduction in total nitrogen. Based on these results, we propose that composting

hydrocarbon polluted soil with the test materials does not improve soil total nitrogen content.

3.6 Total Organic Carbon

Initial total organic carbon (TOC) was high in T₃ compared with T₁, T₂, T₄ and T₅ (Fig. 4). The cause of the high TOC in T₃ was unclear. However, such difference in TOC was expected to occur between T₁ and the other treatments because, T₁ was not polluted with crude oil while the others were. TOC in T₁, T₄ and T₅ were equal and constant in amount during the experiment. The constant TOC in T₁ was acceptable because it was not polluted or composted with any substance. Again, its amount in the treatment was similar to what was reported from control sites by [11,26] in separate studies of crude oil polluted soil. The increase in TOC of T₂ could have resulted from mineralization and release of carbon from the crude oil which was applied. This is supported by Speight [27] who reported 83–87% carbon content from analysis of crude oil. However, the high TOC in T₂ disagreed with field reports of crude oil polluted plots [11,26]. Higher TOC content in T₃ suggested that the composted material contributed to the input of TOC in the treatment. This was shown in 115%, 41% and 29% increases over TOCs of T₂ on the 1st, 30th and 150th days sampling results. TOC was low in T₄ because sawdust soil amendment has no effect on soil TOC [6]. This may be responsible for similar observation in T₅. There were significant differences between treatments T₁ and T₂/T₃.



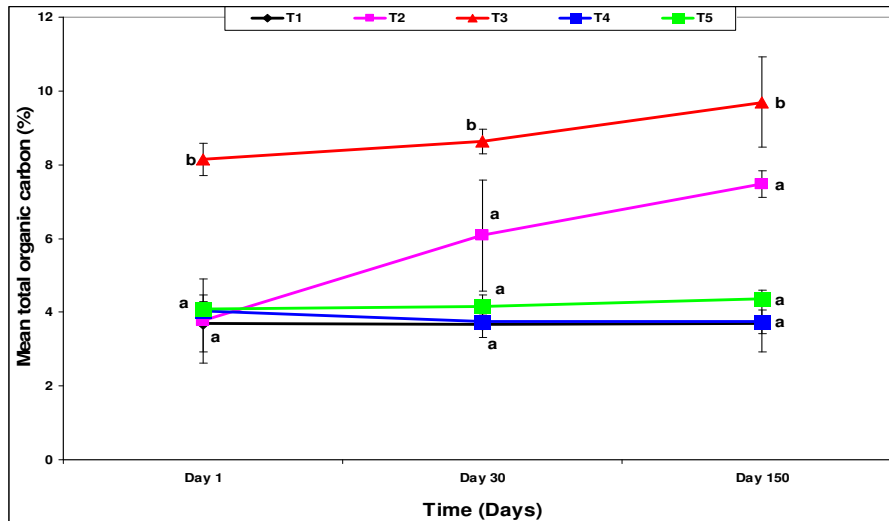


Fig. 5: Effects of the different compost treatments on soil total organic carbon

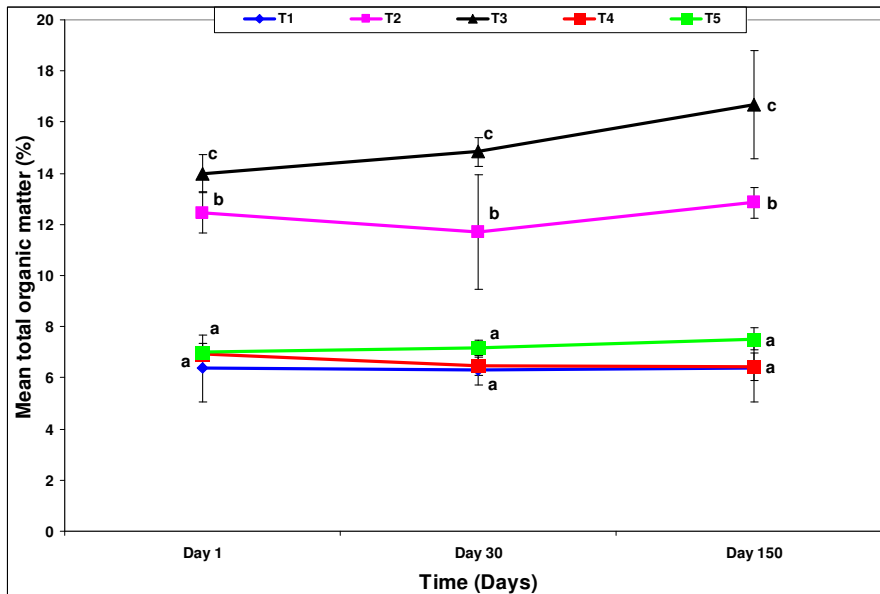


Fig. 6: Effects of the different composting treatments on soil total organic matter

3.7 Total Organic Matter

The effects of composting hydrocarbon-polluted soil with 'spent' mushroom substrate and sawdust on soil total organic matter, TOM was summarized in Fig. 6. TOM of the treatments followed the same trend as TOC. Changes in TOM of treatments could be explained by the same factors that affected TOC. There were significant differences between treatments at $P = 0.05$.

4. CONCLUSION

The capacities of 'spent' mushroom substrate and sawdust to stimulate degradation of petroleum hydrocarbon in crude oil polluted soil were evaluated in pot experiment. The results obtained demonstrated that composting crude oil-polluted soil with 'spent' mushroom substrate stimulated degradation of total petroleum hydrocarbon content of the polluted soil. It also showed that composting with sawdust increased

reduction in petroleum hydrocarbon content of the crude oil-polluted soil. Therefore, we propose that composting crude oil-polluted soil with 'spent' mushroom substrate or sawdust increased degradation of petroleum hydrocarbon content of the polluted soil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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