Indian Journal of Agricultural Sciences **92** (1): 63–9, January 2022/Article
https://doi.org/10.56093/ijas.v92i1.120841

Carbon mineralization potential of non-edible oil-seed cakes at different composting stages in soil

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Received: 01 September 2020; Accepted: 04 August 2021

ABSTRACT

Non-edible oil-seed cakes of neem, madhuca and simarouba were subjected to natural decomposition by simple pit method in CR design during 2020–21 at UAS, GKVK, Bangalore. The physicochemical parameters temperature, pH, EC, mineral nutrients, lignin contents and phytotoxicity of oil-seed cakes during decomposition were determined at 30 days intervals for 90 days. Simultaneously, samples were also studied for the C-mineralization pattern by measuring CO_2-C evolution during 60 days of the aerobic incubation experiment. The first-order kinetic model was used to describe the C-mineralization and calculate potentially mineralizable C. The decomposition of oil-seed cakes led to an increase in mineral nutrients and a decrease in lignin content and toxicity. After 60 days of the addition of soil with oil-seed cakes at different stages of compost, the cumulative C-mineralization occurred in the order neem<madhuca<simarouba. The kinetic model suggested the presence of potentially mineralizable C (C_0) in the undecomposed simarouba oil-seed cake than in the neem and madhuca. This C_0 decreased at 90 days of composting in all oil-seed cakes. The NMC was high in undecomposed oil-seed cakes in the order S-0>M-0>N-0. The CMC value of mature compost (90 days) was lower in neem and simarouba (6%) than in madhuca oil-seed cakes (22%). The low C-mineralization potential of raw and composted neem oil-seed cake suggested its superiority in the improvement of SOC. However, complete composting of oil-seed cakes of madhuca and simarouba was necessary for C-sequestration.

Keywords: Carbon mineralization, First-order kinetic model, Madhuca, Neem, Oil-seed cake composting, Simarouba

Carbon mineralization has been widely used to determine the organic material mineralization in the soil under controlled conditions. Hence, C mineralization as measured by CO_2 evolution or microbial respiration is used as a general indicator of the persistence or decomposability of organic materials applied to soil (Anderson 1982, Wang *et al*. 2020). The non-edible oil-seed cakes, a byproduct of biofuel industries, are a good source of carbon post degradation. Large quantities of oil-seed cakes produced from the non-edible oil-seeds of neem, pongamia, jatropha, simarouba, madhuca and castor (Yadav *et al.* 2011) are a good source of lignocellulose and organic matter (OM). This has generated a lot of interest in its utilization and application as a soil organic amendment (Lopes *et al.* 2009, Das *et al.* 2011). Oil-seed cakes require appropriate treatments to avoid harmful phytotoxic effects which ultimately lead to inhibition of plant growth (Sahoo *et al.* 2010, Malinska *et al*. 2017). The most common remedy for this purpose is composting, a biological process of aerobic decomposition that degrades the labile organic matter and generates CO_2, inorganic nutrients and stable organic materials containing humic-like substances (Srinophakun 2012, Sayara *et al*. 2020).

The process of C-mineralization of different organic wastes after the soil amendment has been studied previously (Masunga *et al*. 2016, Salvator *et al*. 2019, Wang *et al*. 2020), however, there is no study on the decomposition and C-mineralization of certain non-edible oil-seed cakes in soil. In this regard, small-scale and short-term experimental methods involving incubation of the soil and oil-seed cake composting mixture were carried out. This study helps to elucidate C-mineralization dynamics and the data obtained could be fit to kinetic models (first-order exponential), that allow the determination of potentially mineralizable C and its mineralization rate. This is based on the observation that the accumulation process of the SOC mineralization process during incubation time was consistent with the first-order kinetic model (Wang *et al.* 2020). The present study helps to understand the comparative dynamics and kinetics of C-mineralization in sandy loam soil amended with either raw or composted non-edible oil-seed cakes of neem, madhuca and simarouba.

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MATERIALS AND METHODS

Decomposition set up: The oil-seed cakes of neem (N), madhuca (M) and simarouba (S) (30 kg each) were procured from the Biodiesel Production Unit of Biofuel Park located at Hassan, Karnataka. The composting was done separately by a simple pit method (1 m × 1.5 m × 2 m), with each pit containing a specific oil-seed cake, soil and rice straw mixture in the ratio of 6:1:1 (Wong *et al.* 2001) during 2017–18 at UAS, GKVK Bangalore campus. Each compost mixture was sampled randomly at an interval of 30 days and the subsamples were preserved in a deep freezer (-35°C) for soil incubation experiment. Each sample was subjected to the analysis of the physico-chemical parameters such as temperature (°C), *p*H and electrical conductivity (EC). The organic carbon (OC) was determined by the Official Methods of Analysis Association of Analytical Chemists (AOAC) (Anon. 1995). The total nitrogen was determined by the Kjeldahl method (Krick 1950). The total phosphorus (P) was determined by the vanado molybdate method (Jackhson 2014) and potassium (K) by the Flame photometer method (Singh *et al.* 2007). The total solids (Anonymous 1999), germination index of radish seeds (Zucconi *et al.* 1981) and lignin content were determined.

Carbon mineralization studies: The carbon mineralization study was carried out by soil incubation experiments according to the method of Bernal *et al.* (1998a) with some modification. The whole set was maintained in the anaerobic condition in a closed system. Ten grams of air-dried and sieved (2 mm) top sandy loam (Sand 60%, slit 22%, clay 18%) soil samples (bulk density 1.5 g/ml, *p*H 6.78, N- 1.03%, P-6.07%, and K- 8.96%) were mixed separately with 200 mg portion of compost samples of three oil-seed cakes at different composting intervals (0, 30, 60 or 90 days, equivalent to 48 t/ha). About 1.8 ml distilled water was added to each soil-compost mixture to bring the moisture content to 60% of their water holding capacity and placed in a 500 ml incubation vessel. There were three replicates for each treatment. A separate control without any compost amendment was maintained for each set of treatments. The evolved CO_2 was trapped in 10 ml 0.1N NaOH contained in a tube that was placed near the soil-compost mixture in the closed incubation vessel and incubated in a BOD incubator maintained at 28°C for 60 days. The quantity of CO_2-C released was measured at 0, 5, 7, 9, 12, 20, 25, 30, 40, 50 or 60 days by titrating with 0.1N HCl after precipitating the carbonates with 1.5 M $BaCl_2$ solution. The cumulative CO_2 evolved was calculated for each sample date as mg CO_2-C.

C-mineralization kinetic study: The best fit kinetic model was selected based on dependencies less than 0.98 (Jagadamma *et al*. 2014) and greater R^2 (Farrar *et al*. 2012) using Sigma Plot ver. 14.0.

First-order exponential model (Chaudhary *et al*. 2014) was determined as:

$$C_m = C_0\ (1\text{-}e^{kt})$$

where $C_{0,}$ total potentially mineralizable C (mg/kg); k, mineralization rate constant (per day).

First-order E model (Zhang *et al*. 2017) was calculated as:

$$C_m = C_0\ (1\text{-}e^{kt}) + C_1$$

C_1 (mg/kg) represents a separate pool of easily degradable substrate that produces a mineralization flush during the first incubation interval. C_0 is the size of active C fraction (mg/kg) and k is the decomposition of active C fraction (per day).

C-mineralization parameters: Net mineralization coefficient (NMC) and complementary mineralization coefficient (CMC) were calculated to assess the mineralization capacity of soil in the presence of oil-seed cake compost samples. Both parameters were calculated based on the cumulative carbon evolved as CO_2 after 60 days.

$$NMC = C_A \times 100/(C_C+C_B)$$

$$CMC = (C_A\text{-}C_D) \times 100/\ C_C$$

where C_A= total CO_2- C (Cumulative); C_B = total organic carbon in the soil; C_C is the total organic carbon added with compost; C_D is CO_2-C emitted from the control.

Statistical analysis: The data for physicochemical properties and kinetic parameters were analyzed using XL-Stat (Microsoft 2016). The two-way analysis of variance (ANOVA) was carried out and the means of treatments were compared when the F value was significant (P≤0.05). Duncan's multiple range test (DMRT P≤ 0.05) was used to differentiate between individual means.

RESULTS AND DISCUSSION

Chemical characteristics of oil-seed cake compost samples: The oil-seed cakes of neem, madhuca, and simarouba at different stages of decomposition showed a wide variation in chemical composition (Table 1). The *p*H of all composted samples ranged from 6 to 8. The high C:N ratio was recorded at zero-day old composts, whereas it was reduced at 90-days of composting in the case of all three oil-seed cakes. This could be attributed to the composting period of 90 days that reduced the C content and increased the available N content (Charest and Beauchamp 2002). The high mineral nutrient (N, P, K) content could be related to the mineralization for 90-days of composting in all the oil-seed cakes. The lignin content was higher in madhuca followed by neem and simarouba oil-seed cakes at different stages of decomposition. The reduced total solids (TS), C: N ratio, and phytotoxicity in terms of enhanced GI of radish seeds were indications of maturity (Sellami *et al.* 2008) of 90-day old compost samples of oil-seed cakes.

C-mineralization in soil incubation: The cumulative CO_2 evolution was significantly greater in neem, madhuca and simarouba amended soil than the control soil at 60 days of incubation. This could be attributed to microbial degradation and oxidation of organic amendments in soil. This C-mineralization as measured by CO_2 evolution is used as an indicator of persistence or decomposability of organic materials applied to soil (Wang *et al*. 2020). High

Table 1 Physico-chemical parameters at different intervals (0,30,60 and 90-days) of composts of oil-seed cakes of neem (N), madhuca (M) and simarouba (S)

Compost sample	*p*H	EC	C (%)	N (%)	C: N	Lignin (%)	P (%)	K (%)	TS (%)	GI (%)
Neem										
N-0	6.19[a]	1.18[a]	43[a]	1.25[a]	34.4[a]	21.5[a]	0.02[a]	0.85[a]	95.29[a]	34.83[a]
N-30	7.38[b]	1.86[c]	40.4[b]	1.74[b]	23.21[b]	20.1[b]	0.08[c]	1.45[b]	82.42[b]	65.12[b]
N-60	7.16[b]	2.26[e]	38.7[c]	1.85[c]	20.91[b]	19.8[c]	0.1[e]	1.65[c]	72.51[c]	78.31[c]
N-90	7.08[b]	2.43[f]	36.9[d]	2.16[c]	17.08[c]	18.3[d]	0.19[g]	1.69[c]	66.19[d]	93.12[d]
Madhuca										
M-0	6.74[a]	0.71[a]	49.1[e]	1.09[a]	45.04[a]	31.3[e]	0.02[a]	0.6[a]	94.24[a]	7.8[e]
M-30	8.1[b]	1.32[c]	48.4[f]	1.2[a]	40.3[b]	30.9[f]	0.07[c]	0.8a	91.88[a]	23.27[f]
M-60	7.26[a]	1.68[e]	46.9[g]	1.3[a]	36.07[c]	30.1[f]	0.1[e]	1.4[c]	85.73[b]	78.91[c]
M-90	7.13[a]	1.95[g]	45.9[h]	2.1[c]	21.85[d]	29.8[g]	0.2[g]	2.1[e]	76.63[c]	90.23[d]
Simarouba										
S-0	6.76[a]	0.82[a]	46.8[g]	1.0[a]	46.8[a]	8.5[h]	0.06[a]	0.4[a]	91.56[a]	23.78[f]
S-30	7.16[a]	1.15[c]	41.8[b]	1.2[a]	34.83[b]	7.2[i]	0.08[c]	0.54[b]	83.29[b]	62.49[b]
S-60	7.12[a]	1.36[d]	37.1[c]	1.5[c]	24.73[c]	6.8[j]	0.1[e]	0.65[b]	78.51[c]	83.78[c]
S-90	7.05[a]	1.78[f]	35.1[d]	1.6[c]	21.93[d]	6.3[j]	0.2[g]	0.8[b]	72.15[d]	95.76[d]

Different letters in the same column indicate significant differences according to DMRT ($P<0.05$).

C-mineralization occurred initially in the undecomposed simarouba (S-0) and 30-days old neem (N-30) and madhuca (M-30) oil-seed cake composts (Fig 1). This could be due to the presence of easily degradable C in the fresh oil-seed cakes of simarouba. However, in the case of neem and madhuca, the 30-days old composts were rich in highly active C-fraction which could be due to partial degradation of cytotoxic compounds and complex carbohydrates (cellulose, hemicellulose, and lignin) during the early composting process. Initially, the rate of CO_2-C evolution during the incubation experiment increased sharply but declined as time progressed. Similar observations were made by previous workers (Nourbakhsh *et al.* 2006, Wang *et al.* 2020). The zero-day old composts of neem (N-0) and simarouba (S-0) oil-seed cakes (fresh and undecomposed), produced high CO_2-C for 25 and 29 days, respectively. This priming effect could be due to the presence of more bioavailable organic matters in the above oil-seed cakes and this substrate availability is one of the most important factors in organic matter mineralization (Uchida *et al.* 2012). This kind of strong mineralization leads to high carbon loss (Fontaine *et al.* 2007). However, madhuca oil-seed cake (M-0) took a long period (50 days). Subsequently, the C-mineralization decreased with an increase in the composting period in all three oil-seed cakes, before it reached a steady rate. The mature oil-seed cake composts at 60 and 90 days of composting achieved a steady rate earlier (Fig 1). The above observations were also documented in a variety of organic waste applications with varying degrees of maturity in the soil (Bernal *et al.* 1998a). As the readily mineralizable organic components get depleted, relatively recalcitrant organic C portion was also metabolized, resulting in the steady rate of C-mineralization (Rochette *et al.* 2006). This observation was correlated with that of the previous studies (Rodriguez-Salgado *et al.* 2017, Wang *et al.* 2020). The total amount of cumulative C-released or C-mineralized after 60 days of soil incubation with the undecomposed oil-cakes of all three oil-seed cakes ranged from 604.2 to 2,255 mg/100 g of soil samples increasing in the order N-0< M-0< S-0. This could be due to the great variability in the occurrence of compounds with different degrees of maturity of oil-seed cake composts (Bernal *et al.* 1998a). The C-mineralization decreased with increase in the compost maturity period. The least C-mineralization was observed in the 90-days composting of neem (N-90) when compared with the same composting period of madhuca (M-90) and simarouba (S-90). High C-mineralization values in the case of madhuca and simarouba cakes could be due to the low degree of maturity and stability in comparison to that of neem at the same composting period. The presence of a high amount of complex sugars such as lignin in madhuca and simarouba oil-seed cakes as compared to neem oil-seed cake could be accounted for high C-mineralization (Table 1). According to Wang *et al.* (2020), C-mineralization is related to hydrolytic and oxidoreductase enzyme activities in the soil. The complex components of oil-seed cakes are shown to resist microbial and enzyme degradation thus requiring a specific group of the microbial enzyme system and a long duration for compost maturation (German *et al.* 2011). Although the 90-days of composting of neem and simarouba oil-seed cakes resulted in the elimination of phytotoxicity and increased mineral nutrient availability (Table 1), they required more incubation time for carbon stabilization. This suggested that curing or carbon maturation of composts is

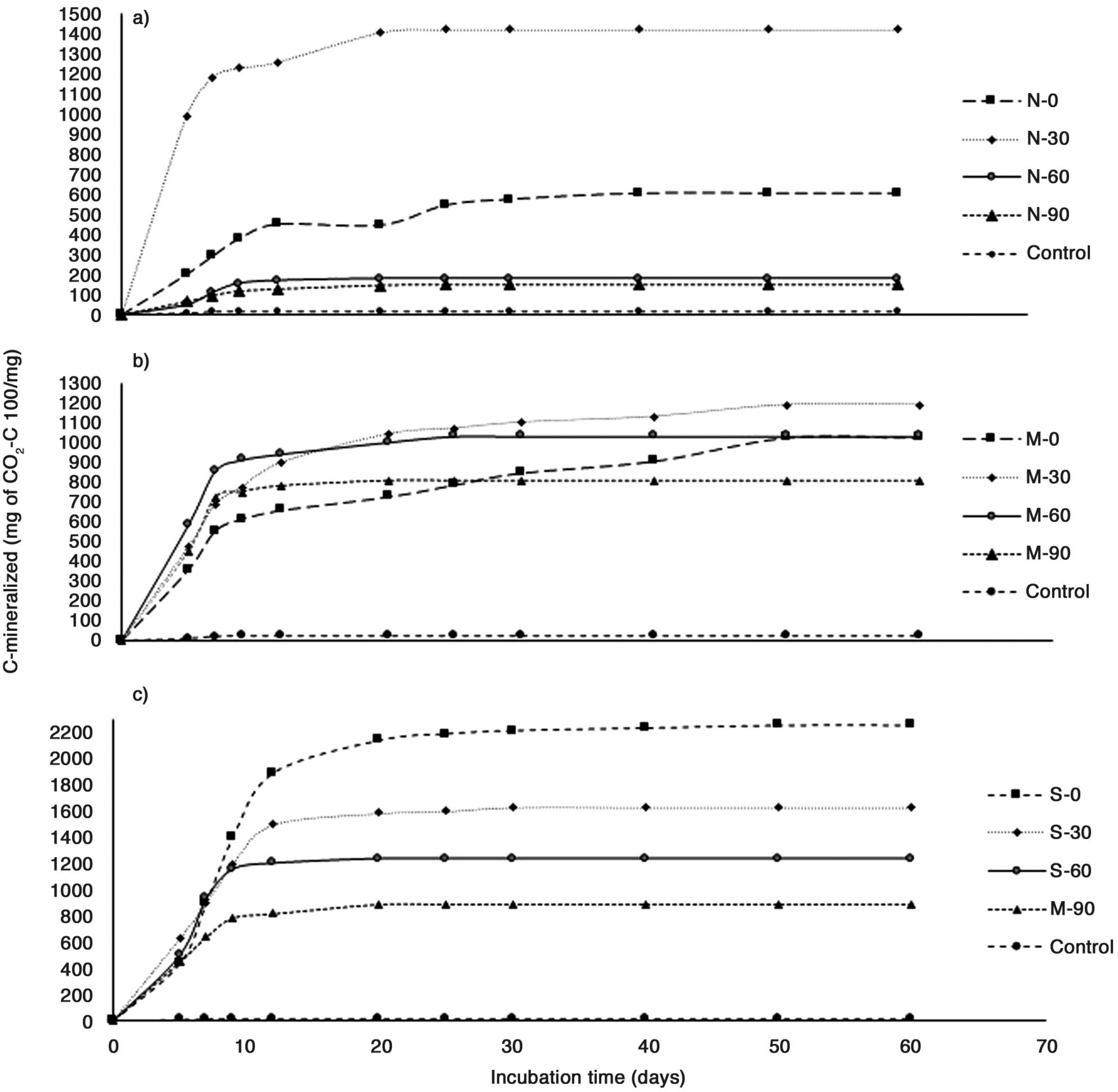

Fig 1 Pattern of C-mineralization at different intervals (0,30,60 or 90-days) of composting of oil-seed cakes of neem (N), madhuca (M) and simarouba (S).

essential before soil amendment.

C-mineralization kinetic parameters: The cumulative CO_2-C evolution data from non-edible oil-seed cakes at different composting periods were used to fit three first-order exponential models. The models were selected based on maximum R^2 values and dependencies of less than 0.98. The data fits into the first-order exponential model very well and fairly to the first-order E model. All results of CO_2-C evolution from non-edible oil-seed cakes (at different intervals of composting) treated soil conformed well to the first-order exponential model $C_m = C_0$ (1-e^{kt}). This model explains the potentially mineralizable C (C_0) and mineralization rate (k). The C_0 value indicates the biologically active fraction of solid wastes which could be released as CO_2-C. The k determines the proposition of organic C mineralized with time. The initiation of the composting of oil-seed cakes irrespective of their source stimulates the microbial activities due to the readily available mineralizable-C residues than the SOC which is relatively resistant to biodegradation. In the present study, the decomposition rate of native soil organic carbon (SOC) in the presence of the amended oil-seed cake samples is presumed to be the same (Wang *et al.* 2020). The rate constant (k) is used to assess the effect of organic residue on soil organic mineralization (Nourbakhsh *et al.* 2006). According to this model, the zero-day-old compost samples of all three oil-seed cakes have low and similar k values (0.09). The initial untransformed oil-seed cakes also have been documented with high carbon and C: N ratio with low k value (Mohmmaed *et al*. 2014). On the other hand, 30, 60 or 90-day composted samples showed high k-value; and in the case of madhuca, it was equal to the control which indicated that the compost samples were mature and that the first step of C- mineralization was very fast. The neem and simarouba oil-seed cake composts have similar k values (Table 2) and indicated the similar decomposability and C-mineralization pattern of the oil-seed composts after soil amendment (Bernal *et al*. 1998a).

Different letters in the same column indicate significant differences according to DMRT (P<0.05)

The neem and madhuca undecomposed oil-seed cake samples had C_0 values 603.7 and 951.6 mg/100 g, respectively. But this value increased 2.2 times in neem and 0.15 times in madhuca through 30-days of composting. This could be attributed to the initial microbial lag phase in the soil, and also the presence of cytotoxic secondary metabolites in untransformed oil-seed cake composting mixtures. At least 30 days of composting were required to remove the toxicity of compost samples and this period increased the C-mineralization. However, in the case of undecomposed simarouba oil-seed cake, high C_0 value (2,3486 mg/100 g) and the associated low C:N ratio and high N concentration (Datta *et al*. 2019) and complex sugar (lignin) contents were recorded. This value reduced gradually by about 1.5 times at 30-days of composting in simarouba oil-seed cake. The C_0 value in all three oil-seed cakes was decreased at the maturity of the compost samples (Table 2). The C_0 value at 90-days of composting was least in neem oil-seed cake (150.5 mg/100 g) when compared to madhuca (814.9 mg/100 g) and simarouba (903.8 mg/100 g) oil-seed cakes. This suggested that the mature composts had the lowest C losses and mineralization (Bernal *et al*. 1998a). The variability in C_0 values of mature compost samples of oil-seed cakes reflected on the differences in oil-cake compost properties.

C-mineralization parameters: The NMC and CMC were calculated to assess the mineralizing capacity of soil in the presence of all three oil-seed cake compost samples. The NMC of sandy loam soil (control) equals one (Table 2), due to SOM associated with sandy soil which is highly susceptible to decomposition. The addition of undecomposed neem and madhuca oil-seed cakes contributed to low NMC value when compared with 30-days of composted samples; then the values again decreased at 60 and 90-days of composting in the above samples. However, undecomposed simarouba oil-seed cake amended soil showed a high NMC value which gradually decreased as the age of the compost increased. The NMC value of mature (90 days) neem oil-seed cake compost was low and similar to the control soil which indicated that the addition of this compost makes a small contribution of organic matter and similar to SOM. The small value of NMC is the indication of the stability of the compost and the high values observed in immature compost (zero and 30 days of composting) samples indicated an important loss of organic C, possibly due to the intense microbial degradation activity of the abundantly available labile organic fraction. According to the CMC results, only around 2% of organic carbon from N-0 had been mineralized which was recorded as a minimum as compared to other compost samples. This might be due to the presence of toxic compounds that retarded the microbial activity. But 30-days of composting of neem oil-seed cake mineralized around 56% of organic carbon, after 60 days of soil incubation.

Table 2 Model parameters of control soil and Net mineralization coefficient (NMC) and complimentary mineralization coefficient (CMC) of soil amended with compost samples of neem, madhuca and simarouba oil-seed cake at a rate of 48 t/ha at different intervals (0, 30, 60 and 90 days)

Composting sample	C_0	k	C_0k	C_1	NMC (mg CO_2-C 100/mg sample)	CMC (%)
Control	20[a]	0.21[a]	4.2[a]	0.00047[a]	1[a]	-
Neem						
N-0	603.7[b]	0.09[b]	54.3[b]	0.01[b]	2[b]	2.007[a]
N-30	1415.7[c]	0.13[c]	184[c]	0.4[c]	5.56[c]	56.17[b]
N-60	185.2[d]	0.15[d]	27.7[d]	1.03[d]	1.42[a]	9.12[c]
N-90	150.5[e]	0.16[d]	24[d]	0.3[c]	0.9[a]	7.09[c]
Madhuca						
M-0	951.2[f]	0.09[b]	85.6[e]	4.1[e]	3.6[d]	36.36[d]
M-30	1163.5[g]	0.11[e]	127.9[f]	0.29[c]	6.04[e]	61.06[e]
M-60	1037.7[h]	0.20[f]	207.5[g]	0.92[d]	5.76[c]	38.72[d]
M-90	814.9[i]	0.22[f]	179.2[c]	1.14[f]	5.62[c]	22.31[d]
Simarouba						
S-0	2348.6[j]	0.092[b]	219.7[g]	17.89[g]	7.7[f]	77.41[e]
S-30	1668.2[k]	0.12[c]	200[g]	5.71[e]	6.71[g]	67.09[e]
S-60	1265.2[l]	0.17[d]	215.08[g]	4.30[e]	6.38[h]	12.03[c]
S-90	903.8[m]	0.17[d]	153.6[h]	1.5[f]	6.02[e]	6.11[c]

The mature composts (90 days of maturity) of neem and simarouba oil-seed cakes mineralized only 6% of organic carbon, while madhuca compost at the same period of maturity mineralized around 22% of organic carbon. The high value of organic carbon indicated the requirement of stabilization time for organic matter contents. According to Garcia *et al.* (1992), the CMC of about 2.5% could be considered to possess a sufficiently stabilized organic matter. Fernandez *et al*. (2007) evaluated a composted sewage sludge and thermally dried sewage sludge in the soil where about 3% and 25%, respectively, of organic carbon was mineralized.

The sandy loam soil amended with undecomposed (0-day old) oil-seed cakes of neem, madhuca and simarouba composting mixtures showed a great degree of C-mineralization since the organic matter content in them are not stabilized and the decomposition process occurred in the soil by native soil microorganisms. Meanwhile, the CO_2-C emission of mature oil-seed cake compost-amended soil is less since they were stabilized during the composting process, while the intermediate mature oil-seed cake composts released intermediate C-evolution (30 and 60-days of composting). The low C-mineralization of the oil-seed cake compost samples leads to the production of humus that plays an important role in soil fertility. Meanwhile, the nutrient supply is most closely associated with the active fraction of SOM. Consequently, the active pool of the raw oil-seed cakes provided more nutrients to soil microbes and promoted soil microbial activity. It is better to add immature oil-seed cake compost samples to the soil to activate the microbial mass unless they contain toxic compounds or otherwise it is better to add stabilized compost samples with microbial load and enhanced organic matter content. In conclusion, the addition of raw or partially composted neem oil-seed cake is recommended to increase the SOM as well as to activate microbial population in the soil. On the other hand, the amendment of composted and stabilized madhuca and simarouba oil-seed cake is a good option for C-sequestration in the unfertile soil.

ACKNOWLEDGEMENTS

The first author gratefully acknowledges the financial support from the Department of Science and Technology, under INSPIRE scheme, New Delhi, India.

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