Effects of EM-4 Biofertilizer on CO_2 Evolution and on the Distribution and Quality of Humidified Organic Carbon Fractions in Soil

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***Abstract** : Organic matter is a indicator of soil quality. The study of its quantity, fractions and stability is an important factor in the determination on how sustainable is the soil management. One of the techniques used to improve crop quality is the use of the biofertilizers such as EM-4. As it stimulates the microorganisms activity in soil, it may also modify the rate of CO_2 evolution and affect the distribution of organic C in the humidified fractions. The objective of the present study was to determine the effects of the use of EM-4 on the stable organic C fraction. To this end, experiments were performed to study the organic fractions contained in the activated EM-4 extract; to determine the rates of CO_2 evolution in soil samples with and without the addition of plant residues and EM-4. Quantitative distribution of humidified SOM and the spectroscopic characteristics of FA, HA and humin was also studied. An increase in evolved CO_2 was observed in all treatments that involved EM-4. It also interfered with the formation of humic substances, including structures with a greater conjugation. This characteristic provided greater chemical stability and resistance to degradation by the subsequent action of microorganisms. This confirms the initial hypothesis that the use of EM-4 stimulated microbial activity and consequently the ability of the soil to degrade more rapidly the plant residues.*

Introduction

Most of the soil organic matter (SOM) is formed by the intense transformation of plant and animal residues reaching the surface of the soil into macromolecular substances ranging in color from yellow to dark, of relatively high molecular mass (when compared to biopolymers) and refractory to biological degradation, denoted humic substances. Only a small part of SOM consists of substances of defined biochemical categories such as proteins, sugars, and lipids, which rarely exceed 10% of total SOM.

Humic substances are divided into three fractions according to solubility: fulvic acids (FA) which are soluble both in acid and basic media, humic acids (HA), which are soluble only in basic medium and precipitate in frankly acid medium, and the insoluble residual frankly acid medium, and the insoluble residual fraction denoted humin.

The relative distribution of humidified SOM fractions can be used as an indicator of the quality of the soil system since these fractions reflect the influences of factors such as climate, vegetation, topography, and type and management, among others, on the environment. The better the environmental conditions, the greater the chance of formation of more condensed humic substances (HA) and the greater the value of the HA/FA ratio. The use of the EM-4 biofertilizer could stimulate the microorganisms of soil, a fact that may modify the rate of CO_2 evolution and affect the distribution of organic C in the humidified fractions.

The objective of the present study was to determine the effects of the use of EM-4 on the stable organic C fraction. To this end, the following experiments were performed: Partial characterization of the organic fractions contained in the activated EM-4 extract; Determination of the rates of CO_2 evolution in soil samples with and without the addition of plant residues and with and without the use of EM-4; Determination of the quantitative distribution of humidified SOM and the spectroscopic characteristics of FA, HA and Humin.

Materials and Methods

One hundred grams of soil from the Bt horizon of a red-yellow Podzolic soil were placed in hermetically sealed 2 liter glass bottles and the following treatments were set up, each with four replicates: S- (soil samples used as control), S+ (soil samples with EM-4 added at the dose recommended by technicians of the Mokiti Okada Foundation), 1- (soil sample with dry corn straw added and incorporated at the proportion of 10 t ha^{-1} and addition of EM-4 at the S+ dose), C-(soil sample with a dried corn husk cover at the proportion of 10 t ha^{-1}), and C+ (soil sample with a dry corn husk cover at the proportion of 10 t ha^{-1} and addition of EM-4 at the S+ dose), C-soil sample with a dried corn husk cover at the proportion of 10 t ha^{-1}), and C+ (soil sample with a dry corn husk cover at the proportion of 10 t ha^{-1} and addition of EM-4).

A CO_2 collector (1 mol NaOH L^{-1}) was placed in each vessel. Four vessels with a volume identical to that of the collector were used as blank. The experiment was carried out under laboratory conditions. The CO_2 daily fixed in both the treatment vessels and the blanks was precipitated with $BaCl_2$ and excess NaOH was titrated with a standard HCl solution. The evolution of CO_2 was monitored for 19 days and the experiment was repeated twice. Three liters of an aqueous EM-4 extract were activated with molasses and concentrated to dryness at low temperature. The residue was re-solubilized with methanol and submitted to chromatographic fractionation. The fractions collected with each eluent were submitted to thin layer chromatography using a polarity mobile phase. The products of this separation were analyzed by infrared (IR) spectroscopy to identify the major functional groupings present.

Results and Discussion

In the two replicates of this experiment, an increase in evolved CO_2 was observed in all treatments that involved EM-4 and was more relevant in the treatments to which plant residues were added (Figures. 1 & 2). This confirms the initial hypothesis that the use of EM-4 stimulates microbial activity and consequently the ability of the soil to degrade more rapidly the plant residues.

The evaluations of readily oxidizable carbon (ROC) in soil at 30, 60 and 90 days after application (DAA) of EM-4 indicated that the values always continued to increase, with general means of 9.48 16.35 and 25.28 g C kg^{-1} for 30,60 and 90 DAA, respectively and appeared to have been affected by the biofertilizer.

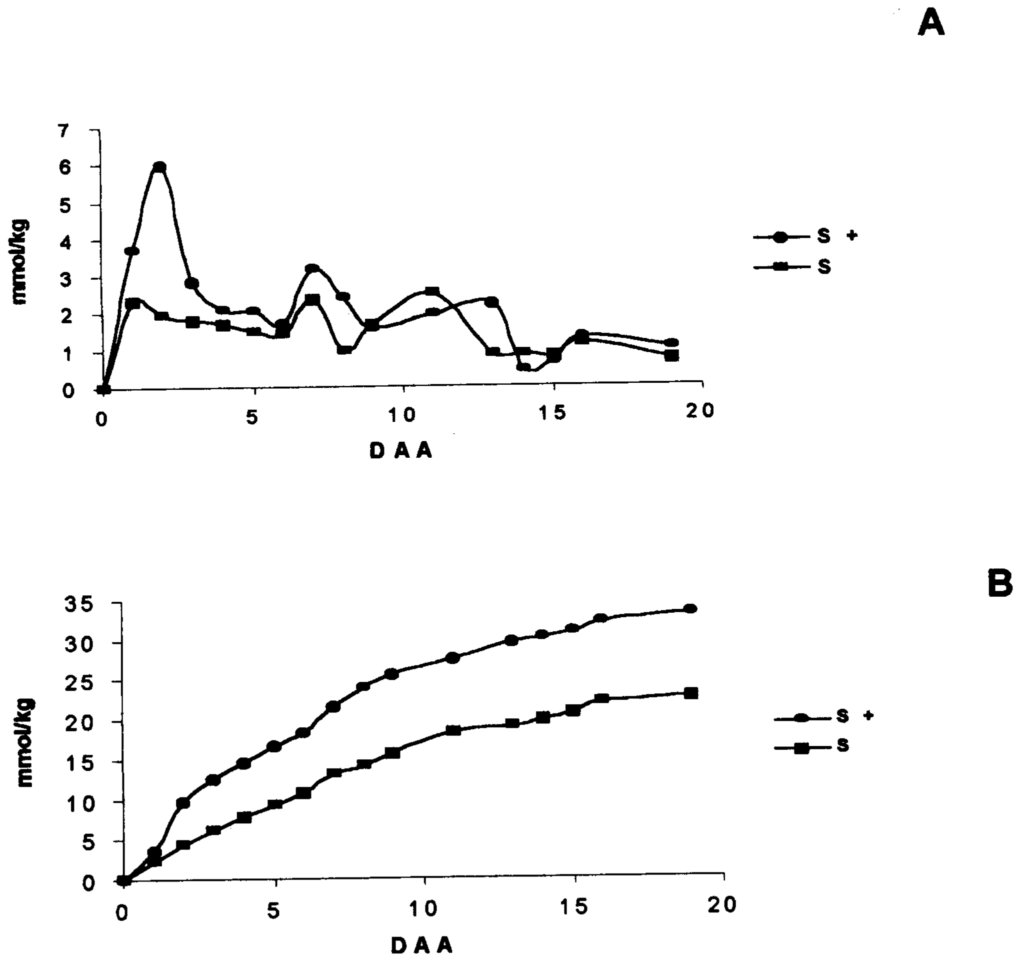
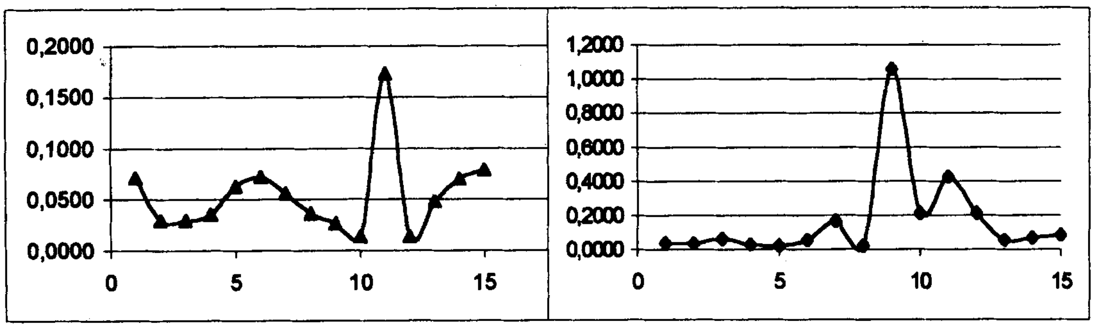
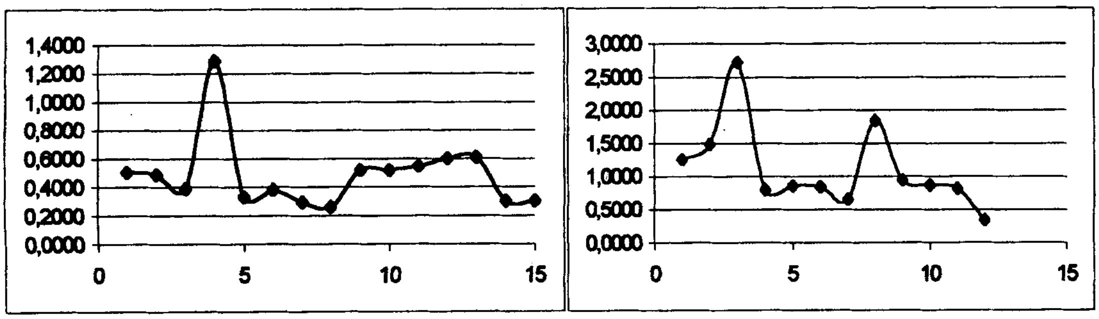


Figure 1. CO_2 **Evolution (mmol** CO_2 **kg**$^{-1}$ **for Control without Organic Matter, with and without EM4 Application (S +). In A daily** CO_2 **Evolution and B Total Amount of** CO_2

CONTROL (No organic matter added)



MAIZE STRAW INCORPORATION (10 t ha^{-1})



MAIZE STRAW - WITHOUT INCORPORATION (10 t ha^{-1})

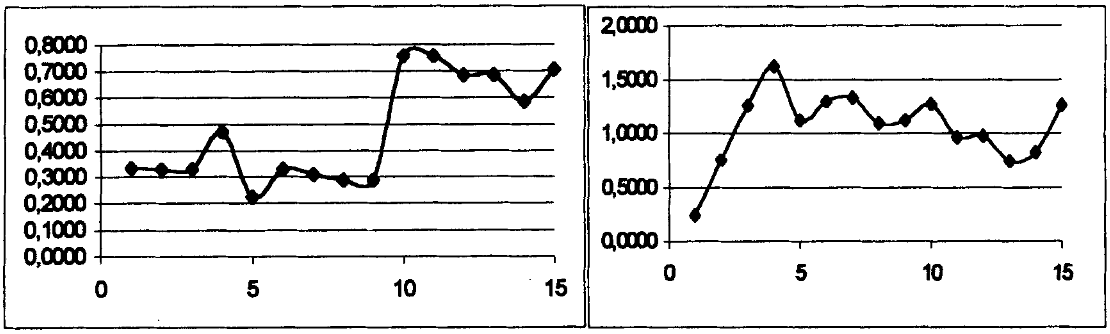


Figure 2. CO_2 Evolution (mgC/g soil) : Left - without application of EM-4; Right with EM (1/100)

The carbon content of FA behaved in a manner similar to ROC (general means of 0.64, 0.80 and 0.90 g C kg^{-1} at30, 60 and 90 DAA, respectively). The carbon present in the FA tended to decrease as a consequence of EM-4 application at 30 DAA when compared to the corresponding treatment without the biofertilizer. This difference was not detected in the other two samplings (60 and 90 DAA). The amount of carbon in FA increased with time, with general means of 0.97, 1.77 and 2.53 g C kg^{-1} over the three samplings.

A tendency to a decrease of the ratio of the absorbances at 2900 cm^{-1} and 1600 cm^{-1} was observed for humic acid and was more marked at 60 DAA. This ratio is an indirect indicator of condensation (a greater content of conjugated double bonds). Similar information was obtained for fulvic acid from the ratio of the absorbances at 1450 cm^{-1} and 1100 cm^{-1}, which was slightly reduced by EM-4 application at 90 DAA.

The evidence presented suggest that EM-4 application interferes with the process of formation of humic substances, inducing structures showing a tendency to greater conjugation. This characteristic provides greater chemical stability and resistance to degradation by the subsequent action of microorganisms.

General Conclusions

1. The application of EM-4 increases the intensity of decomposition of plant residues in contact with the soil. This process favors primary mineralization and thus the availability of nutrient elements to the plants. It would be interesting to conduct more in-depth studies on this topic in order to determine a better synchronization of EM-4 applications and the nutritional demands of different crops, especially short-cycle ones.
2. The application of EM-4 does not significantly affect carbon distribution in the humified fractions
3. The application of EM-4 interferes with the process of humic and fluvic acid formation, inducing structures with a stronger tendency to conjugate, a fact that may favor the stability of these substances in the soil system, improving some of its physical properties.