Effect of EM on Soil Properties and Nutrient Cycling in a Citrus Agroecosystem

D. Paschoal[1], S. K. Homma[2], M. J. A. Jorge[2] and M. C. S. Nogueira[1]
Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, SP, Brazil[1] and
Mokichi Okada Foundation, Ipeúna, SP, Brazil[2]

Abstract

To test the effects of Effective Microorganisms (EM) on soil and on natural cycling of nutrients, a field investigation was conducted with citrus in the State of São Paulo, Brazil. A Typic Hapludox soil predominates as a deep, well-drained, clay soil with high hydrogen and aluminum contents and low base saturation, The organic matter content of the soil is moderate, i.e., about 21 g kg^{-1}. Tested sweet orange trees were of the Pera variety (*Citrus sinensis Osbeck*) grafted on Cravo lemon (*Citrus limonia Osbeck*) rootstock. The design used in this experiment was a Randomized Complete Block Design with 4 replicates for each of the following treatments: 1) control (C), 2) EM applied to soil (EMS), 3) EM applied to citrus plants (EMP), and 4) EM applied to soil and to citrus plants (EMPS). The EM culture used in the experiment was a modified mixture of the three basic cultures, and was sprayed once every 2 weeks. Data presented here are for one season, from March 4 to June 22, 1993, with a total of five applications.

Analyses of the chemical characteristics of the soil and citrus leaves indicate: 1) a significant ($P<0.05$) increase in the organic matter content of the soils treated with EM at both the 0-20 cm and 20-40 cm depths can be attributed to EM's humus forming capacity from dead grass mulch; 2) a significant ($P<0.01$) increase in soil pH at both sampled depths in the EM-treated lots is an indication that EM alone can change soil reactions; 3) a significant ($P<0.05$) increase in soil CEC at both depths in the plots treated with EM can be related to the enhanced soil organic matter formation; and 4) no statistical differences for any of the other chemical parameters in the soil or for the macro- and micronuinents in citrus leaves was found.

No statistically significant differences were found for the physical conditions of the soil, a fact that was almost expected because of the time necessary for full establishment of the microorganisms. Soil compaction tended, however, to be less pronounced for EM-treated plots than for the control.

Introduction

Brazil is the largest citrus producer in the world, and modern technologies have been commonly used by farmers across the country. Agrichemicals continue to be applied which lower the energy efficiency of the production systems and also promote outbreaks of pests and diseases. As sustainable agriculture becomes more acceptable throughout the country, there is a strong tendency to replace synthetic chemicals with naturally-occurring ones. For example, there is a growing interest in Effective Microorganisms (EM) and the use of inoculants of beneficial microorganisms that can improve soil quality and the growth and yield of crops.

Soon after cultures of EM were experimentally introduced into Brazil in early 1989, much attention was devoted to the way in which they affect soils and plants, improve farmlands and increase crop yields and food quality (Anonymous, 1991). The first uses of EM resulted in favorable responses mainly for vegetable and legume crops grown in the Green Belt Area surrounding São Paulo City. In May 1990, the Center for Development of Kyusei Nature Farming was established at lpeúna, São Paulo (also known as the Mokichi Okada Foundation Center) to produce EM and to investigate its properties. The Second International Conference on Kyusei Nature Farming was held at the University of São Paulo College of Agriculture (ESALQ), in Piracicaba, São Paulo, in October 1991.

After the conference, some scientific experiments with EM were conducted by the faculty and researchers from ESALQ and the Mokichi Okada Foundation Center. This paper presents the results of research conducted to evaluate the performance of EM in Brazilian agricultural ecosystems.

EM cultures, as originally developed in Japan (Higa, 1986), consist of mixed cultures of different groups of naturally-occurring species of microorganisms formulated in three basic preparations called EM 2, EM 3 and EM 4 (Higa, 1988). Soil and foliar applications of EM were found to significantly increase the yield and quality of various horticultural and agricultural crops in Japan (Higa, 1986; Higa and Wididana, 1991). Besides Japan, researchers in other countries have also tested EM with such crops as rice in Taiwan (Lin, 1991) and in Myanmar (Myint, 1994); corn in Thailand (Panchaban, 1991); wheat and rice-wheat crop rotations in Pakistan (Hussain et al., 1994); and vegetables in Malaysia (Sharifuddin et al., 1994), Korea (Lee, 1994), Bangladesh (Chowdhury et al., 1994) and Sri Lanka (Sangakkara and Higa, 1994).

Higa and Kinjo (1991) showed an increase in humus content of a soil amended with organic materials and inoculated with EM 4. Although there was little difference in the chemical analyses of the soils among treatments, EM 4 enhanced the growth of cucumber over that of the control and fertilizer-treated plants. The beneficial effect of EM was attributed to the utilization of plant root exudates and the solubilization and mineralization of certain soil nutrients into plant available forms. In other experiments, pure cultures of lactic acid bacteria were unable to promote significant differences in the humus content or chemical analyses of the soil.

Higa and Wididana (1991) concluded that EM cultures can act to 1) induce a soil to become disease-suppressive, zymogenic and synthetic in nature; 2) accelerate the mineralization rate of organic materials added to soil, so as to release nutrients, amino acids and other organic compounds for uptake by plants; 3) increase the number of photosynthetic bacteria and nitrogen-fixing bacteria on both soil and leaf surfaces. All of these could contribute to the growth of more vigorous plants, higher plant yields, and improved crop quality. It is believed that these beneficial microorganisms enhance the plant's photosynthetic rate and efficiency.

There are many unanswered questions relating to the performance of EM in agroecosystems and very few investigations have been conducted on perennial crops. Thus, it was decided to establish a long-term field trial in Brazil to determine the effects of EM on soil productivity and growth of citrus since it is a major crop in the country. Some preliminary observations were made at a nearby citrus orchard that had been in decline, and where the farmer had treated the plants and soil with EM. The effects were dramatic; the EM-treated plants soon began to grow and have since been restored to economic production.

The only other existing study on citrus plants was made by Higa (1988) on Unshu oranges in Japan. By applying EM 2 and EM 3 to groves where soils were previously amended with compost, yields were increased to levels similar to, or even greater than, those of conventional groves where only chemical fertilizers and pesticides were used. EM-treated orchards were almost disease- and pest-free, the leaves were thick and erect, fine roots were numerous, and the soil was soft and well-drained with a large water-holding capacity (Higa, 1988).

Materials and Methods

The site of the experiment is a 17-hectare commercial citrus orchard located in Limeira County, in the State of São Paulo, Brazil. The climate consists of a dry period in the fall and winter seasons (April to September) and a wet period in spring and summer (October to March). Annual precipitation averages 1,408 mm; the driest month is July, with less than 30 mm of rain, and the wettest month is December, when it may rain more than 250 mm (Nascimento and Pereira, 1988). Soil moisture deficits occur from June until September. Relative humidity is between 62 and 77 percent. The average temperature varies from 11^{o}C in the winter to 29^{o}C in the summer. Temperature extremes recorded for this area were -0.6^{o}C and 37^{o}C. The soil is a Typic Hapludox (dark-red Latosol), which is a deep, well-drained clay (Oliveira et al., 1982). Chemical analyses (Tables 1 and 2) indicate that the soil is naturally acidic with a high aluminum content, low base saturation, and organic matter content. The nutrient content is normally low to moderate except for iron which is related to the nature of the parent material. The topography of the terrain is gentle with an approximate 6 percent slope.

Table 1. Effect of EM on Soil Chemical Properties at a Depth of 0-20 cm after Five Applications of EM to Soil (EMS), to Citrus Plants (EMP), and to Both Soil and Citrus Plants (EMPS).

Soil Properties	C (control)	EMS (soil)	EMP (plant)	EMPS (plant-soil)
OM (g kg^{-1})	20.1	23.3	24.1	24.4
pH ($CaCl_2$)	3.2	4.8	5.3	5
P(resin)(mg dm^{-3})	3.7	5.8	3.5	3.8
K ($cmol_c$ kg^{-1})	0.3	0.4	0.3	0.3
Ca ($cmol_c$ kg^{-1})	0.8	0.8	0.9	0.7
Mg ($cmol_c$ kg^{-1})	0.3	0.4	0.4	0.4
H+Al ($cmol_c$ kg^{-1})	5.3	5.5	6.9	6.2
SB ($cmol_c$ kg^{-1})	1.4	1.6	1.6	1.4
CEC ($cmol_c$ kg^{-1})	6.7	7.1	8.3	7.9
V (%)	20.9	22.5	19.3	17.7
B (mg kg^{-1})	0.5	0.6	0.5	0.6
Cu (mg kg^{-1})	3.8	3.8	3.1	4.4
Fe (mg kg^{-1})	221	211	206	222
Mn (mg kg^{-1})	16.8	18.7	14.5	20.7
Zn (mg kg^{-1})	1.0	0.9	0.8	1.2

SB = Sum of Bases
V = 100 SB/CEC (soil Base Saturation)
EM was applied 5 times at 2-week intervals from March 4 to June 22, 1993. At each application, the control plots were sprayed with a volume of water equal to the volume of solution applied to the EM-treated plots after dilution. soil cores were taken from each plot at a depth of 0-20 cm and mixed thoroughly to provide a composite sample for chemical analyses. The data represent the least squares means for each treatment.

Table 2. Effect of EM on Soil Chemical Properties at a Depth of 20-40 cm after Five Applications of EM to Soil (EMS), to Citrus Plants (EMP), and to Both Soil and Citrus Plants (EMPS).

Soil Properties	C (control)	EMS (soil)	EMP (plant)	EMPS (plant-soil)
OM (g kg^{-1})	16.8	20.4	19.4	19.9
pH ($CaCl_2$)	3.3	5.2	5.1	5.8
P (resin)(mg dm^{-3})	1.8	1.7	1.7	1.5
K ($cmol_c$ kg^{-1})	0.3	0.3	0.3	0.3
Ca ($cmol_c$ kg^{-1})	1.0	0.9	1.0	1.1
Mg ($cmol_c$ kg^{-1})	0.5	0.5	0.6	0.6
H+Al ($cmol_c$ kg^{-1})	4.4	5.7	5.7	6
SB ($cmol_c$ kg^{-1})	1.8	1.7	1.9	2
CEC ($cmol_c$ kg^{-1})	6.2	7.2	7.4	8
V (%)	29	23.6	25.7	25

SB = Sum of Bases
V = 100 SB/CEC (Soil Base Saturation)
EM was applied 5 times at 2-week intervals from March 4 to June 22, 1993. At each application, the control plots were sprayed with a volume of water equal to the volume of solution applied to the EM-treated plots after dilution. Soil cores were taken from each plot at a depth of 20-40 cm and mixed thoroughly to provide a composite sample for chemical analyses. The data represent the least squares means for each treatment.

The orchard is five years old. Sweet orange trees are of the Pera variety (*Citrus sinensis* Osbeck) grafted to Cravo lemon (*Citrus limonia* Osbeck) rootstock. Trees are spaced 6 by 4 m (417 plants/ha). Fertilizer, lime and pesticides have not been applied to the soil or plants for the past two years. The previous weed control system consisted of a combination of herbicide sprays under the tree tops and mechanical weeding with the aid of a mower or a light disc harrow between the trees. Fruits are normally harvested in August. The average yield per tree is 40 kg (17 Mg ha^{-1}). Chemical analyses of citrus leaves (Table 3) indicate nutrient deficiencies for calcium, sulfur, zinc and phosphorous; all other nutrients are adequate.

The experimental design was a Randomized Complete Block Design (RCBD) with 4 replicates for each of the 4 treatments. Each plot in each of the 4 blocks has 32 orange trees from which only the 10 central ones were treated; all others were untreated to provide natural barriers against cross contamination from the treated plots. The experimental site is about 18,000 m^2 with a total of 736 trees, 160 treated (40 trees per each treatment).

To assess the homogeneity of the terrain and of the plants within each plot prior to EM application, chemical analyses of the soil and orange tree leaves were conducted on March 4, 1993. After five EM applications, soil and leaves were analyzed again on June 22, 1993.

Table 3. Effect of EM on Chemical Characteristics of Citrus Leaves, after Five Applications of EM to Soil (EMS), to Citrus Plants (EMP), and to Both Soil and Citrus Plants (EMPS).

Citrus Leaf Characteristics	C (control)	EMS (soil)	EMP (plant)	EMPS (plant-soil)
N (g kg^{-1})	25.4	28.7	27.2	27
P (g kg^{-1})	1.2	1.2	1.1	1.2
K (g kg^{-1})	14.3	15.4	12.5	14.5
Ca (g kg^{-1})	28.6	26	26.6	27.3
Mg (g kg^{-1})	3.3	3.1	3.1	3.2
S (g kg^{-1})	0.9	1.4	1.2	0.8
B (mg kg^{-1})	50.9	49.3	45.6	41.9
Cu (mg kg^{-1})	16.1	17	17.2	16.8
Fe (mg kg^{-1})	75.1	115.5	98.4	90.3
Mn (mg kg^{-1})	48.6	50	47.1	48.8
Zn (mg kg^{-1})	17.4	16.7	17.4	17.1

EM was applied 5 times at 2-week intervals from March 4 to June 22, 1993. At each application, the control plots were sprayed with a volume of water equal to the volume of solution applied to the EM-treated plots after dilution. Leaf samples were taken from branches bearing no fruits. The data represent the least squares means for each treatment.

Soil samples were taken from two depths (0-20 cm and 20-40 cm) with a soil auger. Three single soil samples were taken from the surveyed area under the canopy of each plot and mixed throughly to provide a composite sample for chemical analyses. Soil compaction, at a determined soil moisture level, was evaluated comparatively for two points per plot on the same day by means of an impact penetrometer (Stolf et al., 1983). Soil density was assessed at depths of 0-4 cm and 20-24 cm, and soil samples for chemical analyses were taken in the same areas.

For the control (C), water was sprayed on the citrus plants at a rate of 5 liters/plant and on the soil at a rate of 0.25 liters m^{-2}. Application to the soil surface was made to cover an area 2.5 x 4 m on each side of a plant so that each plant would get 5 liters (5 Iiters/20 m^2). For EM applied to soil (EMS), the EM solution was diluted 1 : 1000, sprayed on the soil at a rate of 0.25 liters m^{-2} (equivalent to 2.5 ml of EM/m^2). For EM applied to plants (EMP), EM was diluted 1 : 1000, sprayed on the citrus plants at a rate of 5 liters/plant (equivalent to 5 ml of EM/plant, or about 0.20 ml of EM/m^2 of tree surface); the average tree was 2.75 m high, with a radius of 1.70 m. For EM applied to soil and plants (EMPS), EM was diluted 1 : 1000, and sprayed over the citrus plants at the same rate as for EMP, plus EM was diluted 1 : 1000 and sprayed over the soil at the same rate as for EMS.

For treating soils with EM 4 (treatments EMS and EMPS) and with water (control), a tractor-conveyed spraying apparatus was used. For spraying EM (in treatments EMP and EMPS) and water (control) over the citrus plants a hand pistol spray was used so that all four quadrants of the tree were reached.

EM was spray-applied at 2-week intervals for the first five applications except for the second one which was made one week after the first. When the fall applications were made, fruits were fully developed and ripening had just started. Soil moisture was adequate at the time of the applications and thereafter because of unusually heavy rains in April, May and June. The second sampling of soils and leaves was made at the end of the fall season. The next three EM applications were made at monthly intervals during the winter when plants were in a state of physiological dormancy. The third soil and leaf sampling was made in the spring when the plants were in bloom.

The data presented in this paper are for one season, i.e., from March to June 1993.

Results and Discussion

Homogeneity test data on the chemical characteristics of soils and plants prior to the EM applications indicated variations in the macro- and micronutrient levels among the plots in each block. In order to check the possibility that these differences would interfere with the experimental results, all data were statistically analyzed by analysis of covariance applied to the RCBD; covariates considered were the macro- and micronutrient levels found in soils and leaves prior to EM application. For the parameters in which the F-distribution test was significant, a multiple comparison technique through the t-distribution test was used to compare each mean with every other mean (Freund and Littel, 1981).

Data on the chemical characteristics of soils are shown in Tables 1 and 2. The following conclusions were drawn. First, the organic matter content (OM) of the treated soils increased to significant levels ($P<0.05$) at both depths over that of the control. Higher OM levels can be attributed to EM's ability to form humus from a grass mulch. For this soil parameter, the EM treatments were not significantly different from each other. Second, soil pH in the treated plots also was increased to significant levels ($P<0.01$) at both sampled depths which is an indication of an interaction of EM with soil components. Once again, the EM treatments were not significantly different from each other. Third, the cation exchange capacity (CEC) of the soils reached higher levels in the EM-treated plots; the statistical analysis showed the differences to be significant ($P<0.05$) at both depths. The higher CEC can be related to the OM content of the soils which was enhanced by the activities of EM. As for the other soil parameters, the EM treatments were not significantly different from the controls. Fourth, for all other soil chemical properties no statistically significant differences were found.

Data on the chemical characteristics of citrus leaves are presented in Table 3. No statistically significant differences could be detected between the treatments. A possible explanation for this finding might be the stage of physiological dormancy of the citrus trees at the time the experiment was conducted, i.e., in the fall.

Data on the soil physical properties were statistically analyzed according to a RCBD; the F-distribution test was applied to the analysis of variance to test the hypothesis of equality of the treatment means. Tukey's test was only applied when the F-distribution test was significant for the treatment means. Statistical analyses of soil physical properties (Table 4) showed no significant differences among treatments. This finding was not unexpected since very little time was available for the establishment of the microorganisms. In addition, weather conditions were mostly dry at the time of the experiment (dry season), although some unusually heavy rains did occur in April, May and June.

Soil compaction was evaluated with an impact penetrometer and tended to be less pronounced in the EM-treated plots than in the controls. This may indicate that the establishment and activity of EM had begun to improve the soil physical conditions and reduce compaction as reported by Tokeshi and Lima (1993).

Table 4. Effect of EM on Soil Physical Properties at Different Depths after Five Applications of EM to Soil (EMS), to Citrus Plants (EMP), and to Both Soil and Citrus Plants (EMPS).

Soil Depth	Soil Moisture (%)	C (control)	EMS (soil)	EMP (plant)	EMPS (plant-soil)
Compaction (strokes dm^{-1})					
11cm	16.7	5.8	5.5	4.6	5.1
31cm	17.6	4.8	4.7	5.o	4.5
51cm	19.2	5.1	4.1	4.4	3.9
Bulk Density (g cm^{-3})					
0-4cm		1.24	1.2	1.24	1.22
20-24cm		1.36	1.4	1.37	1.4
Macropores (%)					
0-4cm		25.4	26.4	23.9	24.9
20-24cm		20.8	18.1	16.5	17.2
Micropores (%)					
0-4cm		30.1	28.5	30.7	29.2
20-24cm		28.1	31.2	31.9	31

EM was applied 5 times at 2-week intervals from March 4 to June 22, 1993. At each application, the control plots were sprayed with a volume of water equal to the volume of solution applied to the EM-treated plots after dilution. The data presented are the means for treatments on June 22, 1993.

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