Population Dynamics of Effective Microorganisms under Saline Soil Conditions in Thailand

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Abstract

A study on the dynamics of Effective Microorganisms (EM) under saline soil conditions was conducted in laboratory, composting, and pot experiments. The objectives of this work were to examine the microbial populations in EM stock solutions, to evaluate the properties of EM treated composts, and to elucidate the dynamics of EM under saline soil conditions.

The results indicated that the prominent feature of EM2, EM 3, and EM 4 is the presence of significant numbers of actinomycetes, bacteria, and fungi, respectively Physical, chemical, and biological properties of 15-dey fermentation of water hyacinth composts showed no significant differences among treatments. Total microbial populations were highest at the first sampling date and the majority of activities were in the order of magnitude: first > third > second sampling dates. By cumulative summation, EM (1:500) treated compost gave the highest over-all microbial populations. Compost amendments exerted some effects on pH and EC of saline soil. Yield of onion was correlated with the total microbial populations. These data suggest that EM treated compost can be recommended as an efficient soil amendment in ameliorating a slightly saline soil.

Introduction

A technology on the utilization of Effective Microorganisms (EM) in Kyusei Nature Farming has received a vast amount of attention in agricultural development. This is because Kyusei Nature Farming is a system for practicing agricultural production without interrupting the natural ecosystem and without the use of chemical fertilizers and other agricultural chemicals. Its aim is to utilize the natural ecosystem of regional environments in order to ensure pollution-free food products, to conserve energy, to reduce production costs, to make the best use of resources, and to revitalize agriculture in rural areas (APNAN Newsletter, 1990).

Since 1983, a series of practical applications of EM stock solutions to improve the productivity of soils and crops and to improve the quality of water and environment have been studied, developed, and demonstrated internationally (Higa, 1988). Recently, as a result of the First International Conference on Kyusei Nature Farming held in Thailand in 1989, the report and recommendations of the working group indicated the research needs and priorities of EM technology. Studies on the efficient use of microbes to improve soil fertility and plant growth, including research on the use of EM under site specific conditions, were listed as a short term priority (APNAN Newsletter, 1990).

In Northeast Thailand, salinity is one of the most serious soil management problems. These salt-affected soils have moderate to severe limitations on crop production (Pairintra and Sato, 1987). It has been suggested that short-term control can be achieved on slightly salt-affected areas with low cost technology such as agronomic management and organic amendments (Arunin, 1988).

It is, therefore, worthwhile to carefully examine EM technology as a possible remedial measure for alleviating the problem. The present study was conducted to 1) examine the microbial populations in EM stock solutions, 2) evaluate the properties of EM treated composts, and 3) elucidate the dynamics of EM under saline soil conditions.

Materials and Methods

Laboratory Experiment

The objective of this experiment was to examine the microbial populations in EM stock solutions.

An observation trial was performed in the laboratory using the standard dilution total plate count technique (Nair and Subba Rao, 1977). To identify microbial groups, aliquots (0.2 ml) of EM 2, EM 3, and EM 4 stock solutions were transferred into appropriate petri plates of nutrient media (cellulolytic or non-cellulolytic) for culturing bacteria, actinomycetes, and fungi.

Inoculated plates were incubated at 30C for 2 days. Microbial populations are reported as colony forming units per ml (cfu/ml) of the solution.

Composting Experiment

The objective of this experiment was to evaluate the properties of EM treated composts. Water hyacinth was the main component of the composting material. EM treated composts were compared with a microbial-activator product (DLD-1), and conventional compost in an observation trial. Descriptions of the components used in preparing four experimental water hyacinth composts are reported in Table 1. After thorough mixing, the compost piles were placed on vinyl sheets under natural conditions. Daily temperatures of the compost piles were recorded and the piles were turned and mixed at 3-day intervals.

Table 1. Components Used to Prepare Four Experimental Water Hyacinth Composts Including Two Microbial Inoculants.

Component	T2 Conventional	T3 DLD-1	T4 EM(1:500)	T5 EM(1:1000)
Fresh water hyacinth (g)	10,000	10,000	10,000	10,000
Cow manure (g)	500	2,000	500	500
Microbial activator(DLD-1)(g)*	-	30	-	-
EM 2,3,4(1:500)(ml)**	-	-	250	-
EM 2,3,4(1:1000)(ml)***	-	-	-	250
Rice bran (g)	500	-	500	500
Molasses(1:1000)(ml)	100	-	100	100
Rice husk ash (g)	500	-	500	500
Chemical fertilizer (urea)(g)	-	200	-	-

*Microbial population, $13.4x10^7$ to $6.58x10^9$ cfu/g
**Microbial population, 1×10^9 cfu/ml
***Microbial population, 1×10^5 cfu/ml

Pot Experiment

The objectives of this experiment were to elucidate the dynamics of EM under saline soil conditions using four experimental water hyacinth composts (Table 1). For the pot experiment, a Randomized Complete Block Design was employed with two replicates. Treatments were as follows:

T1 = Control (no compost)
T2 = Conventional compost
T3 = DLD-1 compost
T4 = EM(1:500) compost
T5 = EM(1:1000) compost

Seven kilograms of Roi-Et saline variant (Re-sa) soil, a typical Halaquent inland saline soil of Northeast Thailand was used for each pot. The experimental composts were incorporated into each pot with hand mixing at a rate of 1.4 kg/pot. Four bulbs of green onion were planted in each pot and 500 ml of water were applied at 2-day intervals over 45 days. The soils were sampled from the root rhizosphere (Bopalah and Shetty, 1991) at 15-day intervals and counts of bacteria, actinomycetes, and fungi were made by total plate count to evaluate the change in soil microbial populations as previously described. The numbers of microorganisms were expressed on a soil dry-weight basis. The onion plants were harvested at 45 days and growth and yield data recorded. Soil chemical analyses, including pH, EC, total C, total N, organic matter (OM), P_2O_5, and K_2O, were determined (Black, 1965).

Results and Discussion

Laboratory Experiment

Estimates of the microbial populations (Table 2) indicated that EM 2, EM 3, and EM 4 contain large numbers of actinomycetes, bacteria, and fungi, respectively. Some of these populations are higher than previously reported (Higa *et al*., 1984). However, the earlier data did not account for all of the effective microorganisms such as filamentous bacteria and ray fungi which were included in the current estimate.

Table 2. Numbers of Microorganisms in EM Stock Solutions.[1]

Stock Solution	A	Ac	B	Bc	F	Fc	Total
EM2	$1x10^3$	0	$4.3\ x10^7$	$1.8\ x10^8$	$1.0\ x10^2$	$3.0\ x10^2$	$2.2\ x10^8$
EM3	0	0	$3.0\ x10^{11}$	$2.1\ x10^{11}$	$1.0\ x10^3$	$3.0\ x10^4$	$5.1\ x10^{11}$
EM4	0	0	$9.0\ x10^8$	$1.2\ x10^8$	$1.0\ x10^4$	0	$1.0\ x10^9$

[1]A=actinomycetes, Ac=cellulolytic actinomycetes, B=bacteria, Bc=cellulolytic bacteria, F=fungi, Fc=cellulolytic fungi. Numbers are expressed as colony forming units per ml (cfu/ml).

Compost Experiment

Table 3 shows the physical, chemical, and biological properties of the experimental composts after 15 days and just prior to their incorporation into the soil. Properties of fresh water hyacinth are also presented. The physical and chemical properties of the composts were similar except for the DLD-1 compost. That had a somewhat lower phosphorus and carbon content, and a higher pH and C:N ratio. Biological properties such as the C:N ratio of composts ranged from 11.9 for EM (1:500) to 15.7 for DLD-1. It was noted that during composting, the temperature of the DLD-1 compost pile was consistently lower than the other piles, particularly those treated with EM. This may be the result of temperature specific characteristics of the microbial-activator DLD-1 product, whereas the EM-treated piles were influenced by high temperature fermentation mechanisms (Higa, 1991).

Table 3. Physical, Chemical, and Biological Properties of Four Experimental Water Hyacinth Composts After 15 days.

Compost	Moisture (%)	pH	Total C (%)	Total N (%)	OM (%)	C:N	P_2O_5 (%)	K_2O (%)
Conventional	73.85	6.6	0.15	1.96	0.39	12.33	0.73	1.18
DLD-1	73.46	7.0	0.11	1.87	0.37	15.72	0.36	1.17
EM 2,3,4(1:500)	74.93	6.6	0.14	1.75	0.35	11.92	0.84	1.15
EM 2,3,4(1:1000)	74.05	6.5	0.14	1.92	0.38	13.19	0.83	1.25
Water Hyacinth	85.58	6.6	0.09	1.45	0.30	15.00	0.59	0.67

Pot Experiment

Microbial Populations.

Total plate counts of microbial populations in soil 15 and 45 days after being amended with four experimental water hyacinth composts are presented in Table 4. While the microbial populations were somewhat variable throughout, in most cases the numbers of specific microorganisms were higher in soil amended with the DLD-1 and EM-treated composts than in the control and conventional treatments. Another interesting observation was that the numbers of bacteria were higher after 15 days for the DLD-1 and EM composts than for the other treatments, and that there seemed to be little difference after 45 days. This may indicate that the inoculated bacteria had reached a peak earlier than 15 days and were in a steady state of decline. In studies such as this, microbial populations should probably be assessed initially within 8 to 10 days after soil treatment to determine such things as the proper time for reinoculation with the inoculants.

These results are indicative of the dynamic interaction of both indigenous and inoculated microorganisms in soil. Similar trends have been reported by Gomez and Park (1983). Although the figures vary, this finding is in agreement with others who have concluded that soil microbial populations can fluctuate dramatically after the introduction of soil conditioners and microbial inoculants (Nishio and Kusano, 1980). Higa and Arakawa (1987) postulated that it is possible to derive the full effects of the application of cultured microorganisms if the method is appropriate.

Table 4. Plate Counts of Microbial Populations in Soil after Being Amended with Four Experimental Water Hyacinth Composts. [1]

Compost/Treatment	A	Ac	B	Bc	F	Fc
Microbial Populations After 15 Days						
Control	2.3×10^8	2.7×10^8	1.4×10^{10}	4.5×10^{10}	8.0×10^4	2.0×10^5
Conventional	1.7×10^9	1.0×10^9	1.5×10^{10}	1.5×10^{11}	2.6×10^6	2.7×10^5
DLD-1	8.2×10^7	1.1×10^9	1.3×10^{11}	3.4×10^{11}	4.2×10^6	3.3×10^6
EM(1: 500)	3.6×10^8	4.1×10^8	3.3×10^{11}	3.4×10^{11}	2.8×10^6	1.8×10^6
EM(1: 1000)	1.1×10^9	6.1×10^8	1.5×10^{11}	3.4×10^{11}	3.3×10^6	1.3×10^6
Microbial Populations After 45 Days						
Control	2.7×10^9	4.0×10^8	2.7×10^{10}	1.2×10^9	1.3×10^6	1.2×10^6
Conventional	2.3×10^9	1.6×10^8	1.6×10^{10}	7.5×10^9	2.1×10^6	5.2×10^5
DLD-1	1.1×10^7	1.4×10^9	2.6×10^9	1.7×10^{10}	9.9×10^5	7.9×10^5
EM(1: 500)	9.0×10^7	1.2×10^9	8.5×10^9	6.3×10^9	1.6×10^6	1.0×10^6
EM(1: 1000)	3.4×10^8	7.8×10^8	1.5×10^{10}	1.5×10^{10}	4.6×10^6	3.3×10^5

[1]A=actinomycetes, Ac=cellulolytic actinomycetes, B=bacteria, Bc=cellulolytic bacteria, F=fungi, Fc=cellulolytic fungi. Populations are based on total numbers per gram of dry soil.

Soil Improvement.

Early studies by Mori and Pairintra (1989) have shown that compost and mulching in combination with the cut-off root zone technique is an effective means to ameliorate salt-affected areas in Northeast Thailand. Although the soil used in the present study is classified as slightly saline, the compost amendments tended to alleviate the adverse effects of pH and soluble salts in the soil system. Variations in pH level show significant differences among treatments, but the EC values are not statistically different. In terms of the reclamation of salt-affected areas, the reduction of electrical conductivity of the soil extract (EC) to 0.03 dSm^{-1} is considered promising to farmers. Sunathapongsnk *et al*. (1987) also reported that the application of compost amendments tended to increase soil pH. Again the variability in EC and pH of the amended soils indicate the activities of microorganisms in the soil. The beneficial interaction is attributed to the release of organic substances and soluble nutrients (Nishio and Kusano, 1980). Higa (1991) found that when EC exceeds 0.4 dSm^{-1}, the microflora in the rhizosphere begin to change; in particular, the mycorrhizal fungi start to disappear and the activities of microorganisms decline. When EC exceeds 1.0, harmful anaerobic microorganisms become dominant, and various disorders such as the discoloration of plant leaves begins to appear.

Crop Yield.

An interesting result of this study was the highly positive correlation that was shown to exist between the yield of green onions and the microbial populations (r=0.87). The yields of onion were: EM (1:500), 14.6 g/pot; DLD-1, 11.3 g/pot; EM(1:1000), 9.6 g/pot; conventional 8.6 g/pot, and control, 8.1 g/pot. The total microbial populations were: EM(1:500), 7.2×10^{11}; DLD-1, 5.6×10^{11}; EM(1:1000), 5.3×10^{11};conventional, 2.3×10^{11}; and control, 0.9×10^{11}. In addition, plants in the conventional and control treatments were infested with a damping-off disease and some plants were stunted. It is difficult to conclude from this study whether the causes were primarily the result of osmotic or specific ion effects (El-Shinnawi and Frankenberger, 1988), or manipulation of microorganisms in the soil system. However, it is postulated that the EM inoculant or the addition of the DLD-1 microbial-activator product was able to mitigate the toxic effect of salts.

In recent studies, Higa (1991) reported that such systems were under the zymogenic and synthetic processes of soil microbial transformations. These phenomena occur when organic substances in the soil are transformed into inorganic substances through putrefaction and various harmful intermediate products are produced. Synthetic microorganisms such as photosynthetic bacteria can also synthesize amino acids and sugars from these substances using a very small amount of energy.

In other words, they recycle substances into useful organic energy and thereby decrease entropy. It has been reported that zymogenic and synthetic soil prevents infestation by diseases and insects, and pollution of the environment, thus producing high yields of superior crops (Higa *et al*., 1984, 1985, 1986; Higa, 1989, 1991; Panchaban, 1991; Wididana, 1990).

Conclusions

Results of this study are encouraging for future research on EM technology, especially for long-term investigations. Some first-year results have been negative, i.e., for the very strongly acid sulfate soils of Thailand. Nevertheless, members of the Asia-Pacific Natural Agriculture Network (APNAN) are currently conducting research studies to evaluate the reported benefits of mixed cultures of effective microorganisms (EM) used as microbial inoculants in Kyusei Nature Farming. Specifically, they are studying the effect of these microbial inoculants on the decomposition of organic amendments in soils, nutrient release and cycling, soil properties, and on the growth, yield and quality of crops. While somewhat similar experiments are being conducted for comparative purposes, APNAN members are directing their research toward local agro-ecological conditions and the specific farming systems of their own countries.

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Acknowledgements

We gratefully acknowledge financial support from Sekai Kyusei Kyo Thai Kyokai. A special appreciation is extended to Prof. Dr. Teruo Higa for his generous support to the research project. Sincere thanks are due to Dr. J. F. Parr and Dr. S. B. Hornick for providing excellent technical assistance on suggestions and review of the manuscript.