



# PROSPECTS

*Of*

# EM TECHNOLOGY

*in*

# AGRICULTURE

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## 1. Introduction

Increase in agriculture production is the priority need to meet the food requirements of population increasing @ >3% per annum as in case of Pakistan. The excessive use of inorganic fertilizers and herbicides & insecticides for maximizing crop yield resulted in deterioration of physical and biological health of the agricultural lands. This necessitated the inclusion of legume-cereal rotations & green manuring, use of organic manures, leaving fallow lands for the recuperation of physical properties and desirable soil microorganisms for nutrient mobilization and uptake. The situation has been further aggravated with the accumulation of salts in the plough layer in the cultivated lands, reducing the crop yield reasonably. The adaptation of mechanized cultivation reduced the production of farmyard manure as the animals at the farm have been minimized. Generally the farming community is not practicing the cultivation of green manure crops just because of shortage of irrigation water and time interval available between two crops.

The situation has become alarming and it is now imperative to understand the role of soil microflora and fauna in maintaining sustainable crop productivity. Efforts have been made to genetically manipulate rhizobacteria including *Rhizobium* by the Govt. Organizations. The products are being marketed against various names, e.g. Biopower by National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad; Biozot by Pakistan Agriculture Research Council (PARC), and Provincial Research Institutes are also carrying out work on biological fertilization (*rhizobium inoculant*). Experiments with *strains of aspergillus niger* and *penicillium spp* are being conducted to increase solubility of soil phosphorous.

Recently the work done by Prof. Dr. Teruo Higa, University of Ryukyus, Okinawa, Japan is commendable and worth mentioning. He developed the 1<sup>st</sup> batch of Effective Microorganisms, which eventually called EM in 1980. EM is a combination of aerobic and anaerobic species from three main genera: phototrophic bacteria, lactic acid bacteria and yeasts. The application of EM Technology in agriculture has brought revolution in the field of floriculture, horticulture, crop husbandry and land reclamation. Recently the use of EM Technology has helped to control pollution caused by the sewage as well as industrial wastes.

The research carried out in the field of agriculture by using EM Technology is compiled in this document.

## **2. EM Technology**

Prof. Dr. Teruo Higa, Okinawa, Japan wrote two books on “An Earth Saving Revolution” vol-I & vol-II in the year 1993 and 1994 to resolve our world’s problems through Effective Microorganisms (EM). The material given below has been extracted from these two books.

Prof. Dr. Teruo Higa, University of Ryukyus, Okinawa, Japan developed the first batch of Effective Microorganism, which eventually called EM in 1980. It is available in the liquid form. It is produced through a natural process of fermentation and not chemically synthesized or genetically engineered. EM is a combination of various beneficial, naturally occurring microorganisms mostly used for or found in food. EM is a liquid concentrate. It is produced in vats from cultivations of over 80 varieties of microorganisms. The microorganisms are drawn from 10 genera belonging to 5 different families. The most outstanding characteristic of EM is this that it includes both aerobic and anaerobic species coexisting symbiotically in a most beneficially productive manner. EM contains beneficial tiny anabiotic microorganisms form 3 main genera: phototrophic bacteria, photosynthetic bacteria, lactic acid bacteria, yeast, fungi and effective actinomycetes.

### **Photosynthetic bacteria (*Rhodospseudomonas* spp):**

The photosynthetic or phototrophic bacteria are a group of independent, self-supporting microbes. These bacteria synthesize useful substances from secretions of roots, organic matter and / or harmful gases (hydrogen sulphide), by using sunlight and the heat of soil as source of energy. The useful substances developed by these microbes include amino acids, nucleic acid, bioactive substance and sugars, all of which promote plant growth and development. The metabolites developed by these microorganisms are absorbed directly by the plants and act as substrates for increasing beneficial microbial populations. For example, Vesicular Arbuscular (VA) mycorrhizae in the rhizosphere are increased due to the availability of nitrogenous compounds (amino acid) which are secreted by the phototrophic bacteria. The VA mycorrhizae in turn enhance the solubility of phosphates in soils, thereby supplying unavailable phosphorus to plants. VA mycorrhizae can also coexist with *Azotobacter* and *Rhizobium*, thereby increasing the capacity of plants to fix atmospheric nitrogen.

### **Lactic acid bacteria (*Lactobacillus* spp):**

Lactic acid bacteria produce lactic acid from sugars and other carbohydrates, developed by photosynthetic bacteria and yeast. Lactic acid is a strong sterilizing compound and suppresses harmful microorganisms and enhances decomposition of organic matter. Moreover, Lactic acid bacteria promote the fermentation and decomposition of material such as lignin and cellulose, thereby removing undesirable effects of undecomposed organic matter. Lactic acid bacteria have the ability to suppress disease-inducing microorganisms such as *Fusarium*, which occurring continuous cropping programmes. Under normal circumstances, species such as *Fusarium* weakens crop plants, thereby exposing them to diseases and

increased pest population such as nematodes. The use of lactic acid bacteria reduces nematode populations and controls propagation and spread of Fusarium, thereby inducing a better environment for crop growth.

### **3 -Yeast (*Saccharomyces spp*)**

Yeasts synthesize ant microbial and other useful substances required for plant growth from amino acid and sugars secreted by photosynthetic bacteria, organic matter and plant roots. The bioactive substances such as hormones and enzymes produced by yeasts promote active cell and root division. These secretions are also useful substrates for Effective Microorganisms such as Lactic acid bacteria and Actinomycetes.

### **EM as “co existence and co prosperity”**

The different species of Effective Microorganism (Photosynthetic and lactic acid bacteria and yeast) have their respective function. However, photosynthetic bacteria could be considered the pivot of EM activity. Photosynthetic bacteria support the activities of other microorganisms in EM. However, the photosynthetic bacteria also utilize substances produced by other microbes. This phenomenon is termed “Co existence and Co prosperity”. The enhancement of population of EM in soils by application promotes the development of existing beneficial soil microorganisms. Thus, the micro flora of the soil becomes abundant; thereby the soil develops a well-balanced microbial system. In this process soil specific microbes (especially harmful species) are suppressed, thereby reducing microbial diseases that cause soil borne diseases. In contrast, in these developed soil, the Effective Microorganisms maintain a symbiotic process with the roots of plants within the rhizosphere.

Plant roots also secrete substances such as carbohydrates, amino and organic acids and active enzymes. Effective microorganisms use these secretions for growth. During this process, they also secrete and provide amino and nucleic acids, a variety of vitamins and hormones to plants. Further more, EM in the rhizosphere co exist with plants. Therefore, plants grow exceptionally well in soils, which are dominated by Effective Microorganisms.

EM is a living entity containing active microbes. Manufacturing of EM requires good quality water free of pollutants or chemicals. EM can be stored in a closed container for a period up to 6 months if kept in a dark cool place (Refrigeration is not required). EM always has a sweet sour smell. One may notice a white film on the surface of EM solution when it is stored. This is yeast and does not cause any harm to the EM.

The soils having a high population of disease causing microbes (Fusarium) are called Disease inducing soils. These are generally hard and physical characteristics are not conducive for crop growth. The soils having organisms such as *Penicillium*, *Trichoderma*, *Aspergillus* and *Sterptomyces*, which produce antibiotics, are called disease suppressive soils. These soils have very good

physical characteristics. The soils containing zymogenic organisms such as Lactic acid bacteria and yeast are called zymogenic soils. When raw organic matter with high nitrogen contents is applied, the soil develops an aromatic smell, the population of fermenting fungi such as *Aspergillus* and *Rhizopus* increases. These soils have very good physical characteristics with a high water holding capacity.

EM is a versatile product that uses microorganisms found in all ecosystems. The principle of EM is the conversion of a degraded ecosystem full of harmful microbes to one that is productive and contains useful microorganisms. This simple principle is the foundation of EM Technology in agriculture and environmental management.

### **3. Research completed on various aspects of Agriculture**

The research work done by various scientists in different countries in the field of agriculture using EM Technology is summarized below.

#### **1. Ahmad, R. T. Hussain, G. Jilani, S.A. Shahid, S. Naheed Akhtar, and M.A. Abbas,:**

Use of effective microorganisms for sustainable crop production in Pakistan. Proc. 2<sup>nd</sup> Conf. On Effective microorganisms (EM). Nov. 17-19, 1993, Saraburi Thailand, pp 15-27.

Laboratory and field studies were conducted to study the role of EM for sustained crop production in Pakistan. In preliminary studies on rice, wheat, cotton, maize and vegetable, it was found that EM could not produce crop yield at par with chemical fertilizers. However, EM treated plots showed much higher yields than non-EM treated plots in all crops. Application of EM caused 9.5% increase in the yield of paddy and 27.7% increase in the yield of seed cotton. Relatively higher yield of the maize was obtained when EM 2 and EM 4 were applied in combination. A positive response of EM in solubilization of organic phosphorus was also observed in a laboratory study. The root growth enhanced as the availability of P was increased. These studies proved a definite role of EM in enhancing the fertility and quality of soil.

#### **2. Filho, S.Z., R.R. Medeiros, and S. Kinjo:**

Influence of EM on organic matter decomposition in soil under controlled conditions. Proc. 3<sup>rd</sup> Intl. Conf. on Kyusei Nature Farming. Oct. 5-7 1993, Santa Barbara, California U.S.A., pp 242 – 243.

A laboratory study was conducted under controlled conditions to determine if EM could accelerate the decomposition of organic amendments (organic fertilizer) and enhance the recycling and availability of plant nutrients. The soil was a lithosol of medium texture. The organic fertilizer / amendment was a mixture of Bengal velvet bean (3.84 gm), Guinea grass (1.97 gm), and a mixture of rice bran, castor bean oil cake, soybean oil cake, and fish meal (0.81 gm). The moisture level was maintained near the maximum water holding capacity of the soil.

The results indicated that EM accelerated the decomposition rate of organic amendments applied to soils, improved certain soil chemical and physical properties and enhanced the mineralization and availability of plant nutrients. The organic fertilizer + EM resulted in the highest concentration of soluble organic matter and soluble sugar such as glucose compared with organic fertilizer alone. This indicates a rapid rate decomposition with EM. The organic matter + EM improved soil aggregation, drainage, and water holding capacity compared with organic fertilizer alone. This suggests that EM enhanced the production of polysaccharides, which are the “binding materials” needed to promote aggregate formation. The CO<sub>2</sub> production in the organic matter + EM treatment was the highest as compared to chemical fertilizer and organic fertilizer (4545, 316, 4158 mg). This indicates that EM enhanced the extent of organic matter decomposition and the decomposition was completed at a rapid rate.

### **3. Higa, T. and S. Kinjo, 1989:**

Effect of Lactic Acid Fermentation Bacteria on Plant Growth and Soil Humus Formation. First International Conference, Kyusei Nature Farming pp 140 – 147, Oct 17-21, 1989.

A study was conducted to determine if lactic acid bacteria, when inoculated in soil amended with organic materials, could enhance decomposition and the release of plant nutrients, and increase soil humus formation. For this purpose a green house pot experiment was conducted in 11/1988. Soil was mixed with chopped sore grass at a rate of 40 Mg ha<sup>-1</sup>. Mustard seeds were sown at 0,1,2,3 & 5 weeks after the addition of organic amendment. A culture of *Lactobacillus plantarum* was applied to the pots each week at dilutions of 1:500, 1: 1000 and 1:2000. Microbial were made at the time of harvest and expressed as number per gram of dry soil. EM 4 dilutions were made form liquid stock culture that contained 1.2 x 10<sup>9</sup> bacteria per ml.

Populations of fungi, lactobacilli, aerobic bacteria, and actinomycetes were generally higher in soil treated with EM 4 as compare to control. Results indicated that EM 4 accelerated the decomposition of organic amendment in soils and increased the release of nutrients for plant growth. The soil humus contents were also increased considerably with EM 4 treatment.

### **4. Higa, T. and G.N. Wididana, 1989:**

Changes in the Soil Microflora Induced by Effective Microorganisms. First ernational Conference, Kyusei Nature Farming pp 153 – 162, Oct 17-21, 1989.

The study was undertaken to know which combination of EM cultures changes specific problem soils into healthier and more productive soils and which combinations of EM can favorably interact with soil microbial communities and promote beneficial relationships between biotic and abiotic factors which enhance the health and growth of plants.

EM 2, EM 3 and EM 4 cultures were classified:

EM 2 is a mixture of more than 10 genera and 80 species of coexisting microorganisms (photosynthetic bacteria, ray fungus, yeast, molds, etc). these were cultured in a liquid medium at pH 7.0 and stored at pH 8.5. the number of microorganisms was  $10^9 \text{ g}^{-1}$ .

EM 3 consists of 90% photosynthetic bacteria, cultured in a liquid medium and stored at pH 8.5. The number of microorganisms was  $10^9 \text{ g}^{-1}$ .

EM 4 consists of 90% lactobacillus spp. and microorganisms producing lactic acid, cultured in a liquid medium at pH 4.5. The number of microorganisms in the solution was  $10^9 \text{ g}^{-1}$ .

The soil was gray upland soil with a pH of 8.3 and was not cultivated for many years. EM cultures were diluted to concentrations of 0.1% from liquid stock media and watered into the soil at two-week intervals. The microorganisms were estimated with standard methods.

The generic analysis of the bacterial flora showed that the number of fermentative bacteria such as Enterobacter, starch-digesting bacteria e.g. Azotobacter and Clostridia, were increased in the EM treated soil. A combination of EM 2,3,4, markedly suppressed the number of Verticillium, Thielaviopsis, and Fusarium fungal species that are destructive soil born plant pathogens. Some the EM cultures significantly increased the population of Trchoderma and Penicillium species that are known to suppress plant pathogenic fungi in soils. Soil physical properties were generally improved with EM treatment. Fungi can bind soil particles into more stable aggregates. Bacteria can synthesize cementing agents in the form of gums and polysaccharides that also help to promote good aggregation Lynch (1981, found that Azotobator Chroococcum, Lipomyces starkeyi, and Pseudomonas spp can promote the stabilization of soil aggregates. Fungal species of the genera Pseudomonas, Mycobacter, Micrococcus, Flavobacterium, Penicillium, Sclerotium, Aspergillus, and other are also known to solubilising insoluble phosphates to plant-available forms.

##### **5. Hong-Gon, R.: EM Technology:**

The concept, development and option in DPR of Korea Research Center for Effective Microorganisms, Pyongyang, DPK Korea.

The agricultural sector of DPR Korea has utilized conventional systems of chemical agriculture for a long period of time. The use of toxic chemical and with problems of climate experienced over the past few years has resulted in reduced productivity of once sustainable systems of agriculture. Furthermore, the lack of sufficient organic matter for compost making has also resulted in the loss of soil quality with time. The DPR Korea thus adopted the technology of effective Microorganisms as a means of reducing the problems of agriculture and enhancing soil fertility.

## **6. Hussain, T.:**

Effect of EM and without organic amendments on the electrical conductivity and PH of brackish water soil science department, Univ. of Agriculture, Faisalabad.

It was a pot experiment. The organic materials used were farmyard manure, green grass, and filter cake of sugar industry, poultry manure, EM and brackish water. Brackish water of EC  $2.25\text{dSm}^{-1}$  and EM of 1% was used. After 3<sup>rd</sup>, 7<sup>th</sup> and 15<sup>th</sup> days of incubation period, extracts were taken and filtered and analyzed for EC and pH.

It was concluded that there was a general trend of decrease in EC. The EC was decreased more where organic material with EM was used. Similarly pH was generally decreased.

## **7. Hussain, T., G.Jillani, and T.Javid:**

Development of nature farming for sustainable crop production with EM Technology in Pakistan. Proc. 4<sup>th</sup> Intl. Conf. on Kyusei Nature Farming. June, 19-21, 1995, Paris, France, pp 71-78.

A large number of field and greenhouse experiments were conducted in Pakistan since 1990 to evaluate the use of EM as an alternative to chemical fertilizer in crop production. One such study was a long term field experiment conducted for 5 years on a rice-wheat rotation with the treatments: control, chemical fertilizer (NPK), green manure (GM), and farmyard manure (FYM), all with and without the application of EM. Results showed that EM increased crop yield and improved soil physical properties, especially when applied with organic amendments.

## **8. Hussain, T, 2001:**

Imperatives for Reorienting Agricultural Production System of Pakistan with Nature Farming and Technology of Effective Microorganisms (Innovations, Results and Technology Transfer) 1993 –2001.

The research work carried out by T. Akbar 1996 and by G. Qasim 1997 on “recycling of municipal liquid waste using EM Technology for domestic use” and on “Recycling of sewage water and industrial effluent using EM Technology” for their MSc Thesis, University of Agriculture, Faisalabad, Pakistan has been referred. EM treatments @ 0.1% were given to 5 samples, one municipal liquid waste, 4 different local industries for four days. The treated samples were analyzed for odor, turbidity, pH, EC, BOD, COD heavy metals concentrations, TDS & TSS. These parameters were reduced with EM treatment. The same was confirmed by G. Qasim 1997. It is reported that EM has the potential to deoxidize the heavy metals and convert it into organo-metallic compounds, which are not harmful for human animal health.

**9. Jamal, T., H. Hasruman, A. R. Anwer, M.S. Saad and H.A.H. Shariffuddin:**

Effect of EM and fertilization on soil physical properties under sweet potato cultivation. Paper presented at the 6<sup>th</sup> EM Technology Conf. Nov. 24-26 1997, Saraburi, Thailand.

A study was conducted to observe changes in soil physical characteristics namely soil texture, bulk density, soil moisture retention and aggregate stability as a result of adding EM to an acid soil (Ultisols) at different fertilization under sweet potato cultivation. The fertilization treatment include inorganic, organic and mixture of organic and inorganic fertilizer each with EM and without EM. Fertilization improved soil aggregate stability and EM inoculation further enhanced this property. The increase in aggregate stability was greatest with EM and organic fertilizer and lowest without EM in inorganic fertilizer.

**10. Jamil, M., T. Husain, G. Jilani, and T. Javaid:**

Mechanisms of plant nutrient supply through technology and its reflection in crop production. Proc. 4<sup>th</sup> Conf. on Effective Microorganisms (EM). Nov. 19-22, 1995 Saraburi, Thailand. pp 8-15.

The study was undertaken to test the benefits of effective microorganisms in pots and micro plots with maize and rice as test crops respectively. The effect of different treatments on bacterial population in the soil was also studied. In pot experiment, NPK, FYM and EM 3 were applied alone and in combination. Application of EM 3 significantly improved the plant growth parameter and counts of beneficial bacteria in the rhizosphere. The highest response was obtained with NPK fertilizer followed non-significantly by EM3 + FYM treatment giving 67% and 60% more fresh biomass weights than control respectively. Rice in micro plots was maximum again with NPK fertilizers; however, it was not statistically different to that of EM Bokashi treatment. Application of EM Bokashi increased the number of Azotobacter, Azosprillium, Bacillus and Lactobacillus in the soil that ultimately influenced the plant growth. All these studies revealed a significantly positive effect of EM cultures, particularly when some organic material was incorporated into the soil.

**11. Javaid, T., T. Hussain, G. Jilani, and M.A. Abbas:**

Research and extension activities for the development of EM-Technology in Pakistan. Proc. 4<sup>th</sup> Conf. on Effective Microorganisms (EM). Nov. 19-22, 1995 Saraburi, Thailand. pp 119-131.

The research on the use of EM for crop production in Pakistan is being carried out since 1990. The recent studies include a long-term experiment on rice-wheat rotation. The results showed enhanced crop yield and improved physical characteristics of soil with EM in combination with organic manures.

## 12. Jilani, G:

Utilization of organic amendments and effective microorganisms (EM) to enhance soil quality for sustainable crop production. A thesis submitted to the University of Agriculture, Faisalabad to fulfill the requirements of Ph.D., 1997.

The research carried out and reported in the Ph.D thesis was initiated in 1990 and completed in 1998. The experiments were carried out in the laboratory, green house and in the field. The experiments were statistically designed with CRD or RCBD triplicated, and the data means were compared by LSD at 5% probability level. The results and conclusions drawn from the experiments are summarized below:

1. The microbiological composition of the EM1 culture was very complex, and the microbes belonged to the groups: aerobic and microaerophilic N-fixing bacteria (*Azotobacter*, *Azospirillum*, *Pseudomonas*); photosynthetic bacteria (*Rhodospseudomonas*, *Rhodospirillum*, *Rhodobacter*), P-solubilizers (*Bacillus*, *Aerobacter*, *Xanthomonas*, *Aspergillus*, *Penicillium*, *Candida*, *Streptomyces*); fermenting microbes (*Lactobacillus*, *Mucor*, *Saccharomyces*, *Trichoderma*).
2. The EM<sub>1</sub> has the capacity to fix atmospheric N, solubilize P, decompose organic matter (OM) and produce plant growth promoting substances in a significant amount.
3. More organic C and N were conserved in soil during OM decomposition with EM although the C:N ratio was also lower.
4. Application of EM<sub>4</sub> improved the growth and yield of rice and wheat, and NPK uptake in these crops.
5. The amount/availability of NPK nutrients and OM content in the soil was improved significantly by EM application.
6. The physical, chemical and biological characteristics of soil were rendered with EM in favor of sustainable crop growth.
7. Soil pH, E<sub>Ce</sub>, bulk density and resistance to root penetration were reduced with EM treatments.
8. Application of EM increased the efficiency of GM and FYM, and this combination produced crops yield statistically equal to that with NPK fertilizer alone.
9. Supplementation of EM<sub>3</sub> and EM<sub>4</sub> to half dose of N and P fertilizer respectively, produced similar wheat yield as with their full dose.
10. In EM treated soil the number of N-fixing, P-solubilizing and OM fermenting microorganisms were statistically higher than in no EM respective treatments.
11. In continuous experiments, the EM application showed a linear increase in crop yield and soil improvement with time.
12. The overall value of soil quality index (SQI) was considerably higher with OM + EM treatment as compared to only OM or chemical fertilizer treatments.

## 13. Lee, K.H:

Effect of Organic Amendments and EM on the Growth and Yield of Crops and on Soil Properties. Proc. 2<sup>nd</sup> Intl. Conf. On Kyusei Nature Farming. Oct. 7-11, 1991 pp 142-147.

Several crops were studied in field plots to determine the effect of compost and EM on growth and yield, and on soil chemical properties. Rice yields in the EM +

compost treated plots were similar to those in the NPK plots; the EM + compost treatment may have supplied the nutrients for equivalent productivity. During the growing season with red pepper, both EM solution and EM + compost increased the levels of available  $P_2O_5$ , Ca, and Mg in the soil. After rice harvest, soil in the EM treated plots was found to have a higher  $P_2O_5$  concentration compared with other treatments.

#### **14. Minsk, 1998:**

EM: Effect on plant growth and development, effect on radio nuclide transfer from soil to plants, effect on biological consequences of irradiation in organism. Institute of radiobiology, national academy of the republic of Belarus.

EM-1 introduction into soil before and after sowing plants (Gramineae-oats, Leguminosae-Soya) leads to the activation of photosynthetic processes (which increases the formation of chlorophyll, protein and of activity of number of enzymes, in particular, increase of peroxidase activity with insignificant change all decrease of chlorophyllase activity) in plants. This is an important factor promoting growth and development of plants. EM-1 is able to increase the formation in plants of chlorophyll-green pigment, which takes part in processes of absorption of solar energy, carbon dioxide and other substances and provides the growth and developments of plants. EM-1 increased the formation of protein. EM-1 promotes the activation of peroxidase, which participates in energy related, and protective processes in plants.

#### **15. Minsk 1998:**

Effective Microorganisms: effect on plant growth and development, effect on radionuclide transfer from soil to plants, effect on biological consequences of irradiation in organism. Institute of Radiobiology, National Academy of Sciences of the Republic of Belarus.

In order to estimate the effect of EM on the growth and development of plants the germination, height of plants were studied as well as the length of root and leaf, photosynthetic processes in plants, antioxidant and plastic abilities of plant, crop qualitative and other characteristics. The majority of experiments were held on Gramineae representative-oats, and Leguminosae-soya. Various soils and cultivation conditions were used. The experiments were held in the conditions similar to phytotron (taking into account the presence of winter period in Belarus), in field condition of biological experimental station of NASB, in 30-Km zone of Chernobyl NPP accident (on the territory of Palesse State Radioecological Reserve, Khojniki district, Gomel region), in the economies of Minsk region (Kletsk district). The soil free from radionuclides and the soil brought from 30-Km zone of Chernobyl NPP accident were used in the experiments in phytotron and at the Biological Experimental Station. Different doses and schemes of EM-1 introduction were used in all experiments.

1. E-M -1 introduction into the soil before and after sowing plants (Gramineae – oats, Leguminosae – soya) increased germination and improved root system, promoted growth of oats and soya. It leads to the activation of photosynthetic processes in plants. This is an important factor promoting growth and development of plants.
2. EM-1 is able to increase in plant the formation of chlorophyll (green pigment which takes part in processes of absorption of solar energy, carbon dioxide and other substances and provides the growth and developments of plants), protein and activity of a number of enzymes, in particular, increase of peroxidase activity.
3. Increase of plastic processes takes under the EM-1 effect, in particular – protein formation.
4. EM-1 introduction promotes the activation of peroxides, which participates in energy-related and protective processes of plants.

**16. Paar, J.F., and S.B. Hornic:**

Transition from conventional agriculture to nature farming systems: the role of microbial inoculants and organic fertilizers. Proc. 4<sup>th</sup> Intl. Conf. on Kyusei Nature Farming. June, 19-21, 1995, Paris, France, pp 57-63.

In 1980 “USDA Report and recommendation on organic farming” documented the experiences of farmers who had shifted abruptly from conventional, chemical based agricultural to organic or nature farming systems without chemical fertilizers and pesticides. Among the most serious problems cited were weed and insect infections, and reduced yields. The authors have argued that the successful transition from conventional to organic/nature farming is possible with an improvement in soil quality which can be achieved through the proper and regular addition of organic amendments to optimize soil tilth, fertility and productivity. Through natural processes and selection, these amendments also tend to increase the numbers and diversity of beneficial soils microorganisms, which are vital to the growth, nutrition, and protection of plants. Use of beneficial and effective microorganisms (EM) as microbial inoculants in agriculture was found to be a promising new technology that can improve soil quality and health and increase the growth, yield and quality of crops.

**17. Pairintra, C. and Pakdee:**

Population dynamics of Effective Microorganisms under Saline Soil condition in Thailand. Proc. 2<sup>nd</sup> Intl. Conf. On Kyusei Nature Farming. Oct. 7-11, 1991 pp 164-170.

A study was conducted 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 EM 2, EM 3, and EM 4 is the presence of significant numbers of actinomycetes, bacteria and fungi respectively. 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 alleviated some effects on pH and EC of saline soil. The beneficial interaction is attributed to the release of organic substances and soluble nutrients. Similar results were also found by (Nishio and Kusano, 1980: Fluctuation patterns of microbial numbers in soil applied with compost. (Soil Sci Plant Nutri. 26(4):581-593). Higa (Soil environment and microorganisms, and health of crops. Report by the International Nature Farming Research Center, Atami, Japan, pp 133) found that when EC exceeds  $0.4 \text{ dSm}^{-1}$ , the micro flora in the rhizosphere begin to change; in particular, the mycorrhizal fungi start to disappear and the activity of microorganisms decline. When EC exceeds 1.0, harmful anaerobic microorganisms become dominants, and various disorders such as the discoloration of plant leaves begin to appear. It is suggested that EM treated compost can be recommended as an efficient soil amendment in ameliorating a slightly saline soil.

**18. Piyadasa, E.R., K.B. Attanayake, A.D.A. Ratnayake and U.R. Sangakkara.:**

The role of Effective microorganisms in releasing nutrients from organic matter. Proc. 2<sup>nd</sup> Conf. On effective microorganisms (EM). Nov. 17-19, 1993, Saraburi, Thailand. pp 7-14.

A study was done to evaluate the effect of solutions of EM 2,4 and deionized water in extracting important plant nutrients from 5 organic materials commonly found in the humid tropics. Extracts were analyzed after incubation for N, P and K. The quantities of N & P released from the organic materials were higher when compared to P. The solution of EM 4 extracted a significantly greater quantity of all nutrients from all organic fertilizer tested. In addition greater proportions of all nutrients were extracted from the organic material with low C:N ratios. The results are presented in terms of the possible benefits of using solutions of EM 4 in organic farming system.

**19. Sangakkara, U.R.:**

Impact of Kyusei Nature Farming with Effective Microorganisms on soil property, physiological parameters and yield of selected crop. Univ. of Peradniya, Sri Lanka.

The use of EM with organic matter, especially those with a low C:N ratio improved soil physical and chemical parameters. There was a greater availability of nutrients and also a residual value over a period of two years EM enhanced germination, plant growth and leaf area indices and plant water retention in the organic systems. This culminated in increased yield components and yields.

**20. Sangakkara, U.R.:**

The technology of Effective Microorganisms case studied application. Royal Agricultural College Cirencester, UK.

After giving brief history of EM Technology development, it is cited that EM includes lactic acid bacteria, photosynthetic bacteria, actinomycetes and yeast. For the time being EM Technology is being used in agriculture and environmental management. It has been reported that the EM application increases the release of nutrients from organic matter, enhances photosynthesis and protein activity and better penetration of roots by improving physical properties of soils. Crop residues and animal wastes can effectively be composted with EM Technology; even the city garbage has been composted and converted into biofertilizer. In environments EM application has helped in reducing odor and cleaning sewage water.

**21. Sangakkara, U.R.**

Research on the technology effective Microorganisms in Sri Lanka. Proc. 3<sup>rd</sup> Intl. Conf. on Kyusei Nature arming. Oct. 5-7 1993, Santa Barbara, California U.S.A., pp 138 – 144.

A comprehensive research program was initiated in 1990 and completed in 1993 at two selected locations in Sri Lanka to test the efficacy of EM on four important food crops selected on the basis of their diversity in growth habit and duration, harvested product and response to dry and wet seasons. During the experiments two sources of organic matters were used to study the effect on some soil physical properties with and without EM. Leaves of *Gliricidia sepium* and rice straw, which have different C:N ratio and are commonly available, were used as source of organic matter. In addition, suitable controls were maintained in order to determine the beneficial effect of EM. Yields of legumes were enhanced with EM to a greater extent than non-legumes. The beneficial impact of EM was also greater with *Gliricidia* which had a lower C:N ratio. EM applied to the bare soil also produced some yield stability. The benefits of EM were greater in a wet season, which provided abundant moisture for microbial activity in the soil. EM and organic matter improved the water holding capacity and reduced the bulk density in all the plots. The impact of EM with *Gliricidia* leaves used as organic amendment was most prominent in increasing water holding capacity of the soil. The impact of *Gliricidia* leaves was greater than rice etc. EM applied with organic matter changes the plant rhizosphere into more conducive conditions for supporting plant growth. The benefits became clear over time with changes in the rhizosphere. The organic matter with low C:N ratio increased the efficacy of EM.

**22. Sharifuddin, H.A.H., et al:**

Nature farming research in Malaysia: effect of organic amendment and EM on crop production. Proc. 3<sup>rd</sup> Intl. Conf. on Kyusei Nature Farming. Oct. 5-7 1993, Santa Barbara, California U.S.A., pp 145 – 150.

In 1990, research on nature farming using EM was started in Malaysia. For the past three years research efforts have compared nature farming using organic amendment and EM with conventional farming using chemical fertilizers in the production of sweet corn and leaf mustard. Results indicate that the use of organic amendments, particularly chicken dung, with EM can significantly increase the yield of both crops.

**23. Tokeshi, H., M.J.A. Jorge, A.B. Sanches and D.Y. Harada:**

Interaction between microorganisms, soil physical structure and plant diseases. Paper presented at the 6<sup>th</sup> EM Technology Conf. Nov. 24-26 1997, Saraburi, Thailand.

The areas where the farmers were using green manure and effective microorganisms technology and maintaining their agriculture profitability by controlling erosion with reduced irrigation and suppressed attack by soil pathogens such as sclerotinia sclerotiorum, without using pesticides were selected for study. Soil compaction, basic water infiltration rate, paint infiltration, porosity, and effect of moisture on the production of apothecia of *S. sclerotiorum* were evaluated in such areas. The application of green manure + EM decreased the soil compaction, increased the basic water infiltration rate and porosity of the soil and with this drastically reduced the production of apothecia of *sclerotiorum*.

**24. Van den ham, F.:**

Effect of EM in crop production-case studied from Holland. Agriton, Noordwelve Zuid, Holland.

The use of EM increased productivity of sugar beet significantly. The supply of EM alone to soils generated income from sugar beet, which was similar to that obtained with 50 Kg N/ha and EM Bokashi. The photosynthetic capacity of plants was increased with the application of EM and that resulted in greater yields.

**25. Yamada, K., S. Dato, M. Fujita, H.L. Xu, K. Katase, and H. Umemura:**

Investigations on the properties of EM Bokashi and development of its application technology. Proc. 5<sup>th</sup> Conf. on effective microorganisms (EM). Dec, 08-12, 1996. Sara Buri, Thailand.

Studies were initiated to examine the properties of Bokashi and the mechanisms of its effectiveness in promoting soil quality and crop yields. The results highlight that Lactobacilli and yeasts were present in higher concentration for a longer period when organic matter was mixed with EM rather than with water. A moisture contents of 30% increased lactobacilli and lactic acid, while reducing the pH. Evaluation of the nutritive values showed that Bokashi had a pH of 5.5, conductivity of 4.3 mS, 900 mg/Kg available N in the form of NH<sub>4</sub> and 10 g/Kg P. The addition of organic matter affected the ratio between Actinomycetes and fungi, while EM influenced the ratio between bacteria and fungi. EM promoted

yields of sweet corn and photosynthesis by enhancing root development and activity. The significant beneficial effects of EM could be due to the interactions between beneficial organisms, organic matter and metabolic substances included in EM or its capacity to produce these growth promoters subsequently.

**26. Yamada, K., S.K.M. Fujita, H.L. Xu, K. Katase, and H. Umemura:**  
Investigation on the properties of EM Bokashi and Development of its application technology. 11<sup>th</sup> IFOAM Intl. Scientific Conf. Aug. 11-15, 1996, Copenhagen, Denmark.

It is known from analytic results that EM Bokashi contains a large amount of propagated Lactobacillus and yeast, intermediate substances like organic and amino acids at high concentrations, and 0.1% of mineral N mainly in NH<sub>4</sub> state, and 1% of available phosphorus with a C/N ratio of 10. Effects of EM Bokashi on soil fertility and crop growth might result from two different factors, the organic materials and EM microbes with the produced substances. EM application promotes plant growth, grain yield and the photosynthetic activity of sweet corn by increasing root development and root activity.

**27. Yong Chol, Ko.:**

Enhancing EM activity on low fertility soils-A case study Packam farm, South Pyongan Province, DPR Korea.

EM with organic matter increase soil fertility. Field studies on soil with low humus contents and Ph values highlighted that EM along with 350 Kg of chemical fertilizers increased yields when compared to that with EM alone, further more, yields of rice, corn and wheat were increased by over 100 % when EM was used with 20MT of organic matter / ha.

**28. Zacharia, P.P:**

Studies on the application of Effective Microorganisms in paddy, sugar cane and vegetable in India. Proc. 2<sup>nd</sup> Conf. On Effective microorganisms (EM). Nov. 17-19, 1993, Saraburi Thailand, pp 31-41.

Field experiments were conducted to study the effect of EM 4 on yield components of paddy, sugar cane and Bhenchi and also on the soil nutrient status during 1993 in the union territory of Pondicherry (India). In experiment 1 EM 4 + FYM + NBK gave the maximum yield. It was followed by EM 4 + NPK and FYM + NPK. In experiment 2 EM 4 + NPK gave the maximum yield, followed by NPK alone and EM 4 + FYM.

Available nutrient content of the soil was found to be influenced by the application of EM 4. Nearly 2.2% increase in available N was noticed in the plot treated with NPK + EM 4 when compared to the plot treated with NPK alone. Similar increasing trend was noticed in available P status also. Similarly available

N and P contents were found to be more in the plot treated with EM 4 + FYM, when compared to the control. With regards to K contents, a reverse trend was observed.

**29. Zhao, Q:**

Effect of EM on peanut production and soil fertility in the red soil region of China. Proc. 4<sup>th</sup> Intl. Conf. on Kyusei Nature Farming. June, 19-21, 1995, Paris, France, pp 99-102.

Peanut is one of the most important crops in the red soil region of China. However, yield are relatively low, averaging about 1500 Kg/ ha. A three-year study was conducted to evaluate the effectiveness of EM on soil nutrient transformations, changes in the type and numbers of soil microorganisms, germination percentage, and yield of peanuts. Two treatments were applied : A) organic manure (OM) and B) organic manure and EM (OM + EM).

Application of EM significantly increased the level of soil available nutrients, soil organic matters, total N, and lowered the C:N ratio. Soil microbial populations were 1.5 times higher in the OM + EM treatment than for OM alone. The numbers of bacteria, fungi, actinomycetes and N-fixing microorganisms were higher for the OM + EM treatment compared with OM alone; the application of EM increased peanut germination, yield and total biomass as compared to control.

#### **4. Experiments completed by EMRO, Pakistan on the reclamation of saline-alkali soils using EM Technology**

##### **4.1 Necessity to take-up trial on salt affected lands**

The major part of Pakistan experiences dry climate and agriculturally important area receives less than 250 mm rain fall. Agriculture is only possible on the level plain with artificial irrigation with canals and tube-wells. The total geographical area is 79.61 million hectares, of which 43.69 m ha possesses potential for agriculture. About 16.84 m ha is canal commanded. About 6.7 m ha has been found affected by salinity and alkalinity.

The effectiveness of EM on marginal, medium and good agricultural lands has been established through experiments by growing various crops. Agriculture is not possible on salt affected soils because of the accumulation of salts in the root zone, even on the soil surface, high pH and high exchangeable sodium. The reclamation takes about 2 – 3 years under conventional methods to amend chemical properties and to enrich with microbial population as per requirements of good agriculture lands. Saline soils are reclaimed with excessive use of irrigation water and alkali soils with the application of gypsum. Generally both the types exist in complex form, meaning thereby that income can only be generated after their complete reclamation. Keeping in view the financial constraint faced by the farmers it was planned to carried out experiments on salt affected lands. The field experiments were conducted on loamy and silty clay soils by growing rice crop during the year 2000 and 2003.

##### **4.2 Material used during the trials**

On experimental site arrangements for farmyard manure (FYM) and poultry manure (PM), preparation of EM solutions and Bokashi (rice bran fermented with EM solution) were made before the start of the experiments. Two tones of air dried FYM + PM composted with EM extended per acre (1 acre = 0.4047 ha) applied at the time of preparation of land, 800 lit EM extended made from 40 lit EM-1 (1:1:18; EM-1 : sugarcane molasses : water) per acre applied in 10 equal dozes, 100 kg Bokashi per acre applied 60% at the time of preparation of land, 20% during the tillering stage and 20% at the earing stage, and biweekly sprayings with thousand times diluted EM extended were used for the reclamation of saline-alkali land to obtain reasonable yield during the 1<sup>st</sup> year. Recommendations with respect to quantity of seed (6–7 kg), growing period (20<sup>th</sup> May to 7<sup>th</sup> June), (transplantation period (20<sup>th</sup> June to 7<sup>th</sup> July), number of plants per acre (80,000) and depth of irrigation water at the time of nursery transplantation (2.5 cm to 3.8 cm) of Rice Research Institute, Kalashah Kaku, Agriculture Department, Govt. of the Punjab were followed strictly. Before sowing of nursery the seed was treated with EM extended solution. Neither chemicals for the treatment of seed nor commercial fertilizer were used during the experiment. Seed treatment with EM and EM sprayings substituted the chemical treatment of seeds and insecticides.

### **4.3 Investigations / analytical work carried out**

The investigations with respect to physical & chemical characteristics, and microbiological activity of the soil reclaimed with conventional method of reclamation (CR) and with EM Technology (EM) are detailed below to understand the mechanism of EM.

#### **4.3.1 Characteristics of original soil, CR soil, EM soil, tube-well water and other materials used in the experiment**

The properties of the original saline-alkali, CR and EM-soil are given in table-1. The soil was loam in texture.

##### **4.3.1.1 pH**

pH of the original soil from 9.8 was reduce to 8.8 with CR and to 6.8 with EM indicating that EM has reduce the salts significantly and with that the nutrients up-take by the plants was enhanced as reflected in the crop performance.

##### **4.3.1.2 ECe**

Electrical conductivity of the saturated paste of the soil was also reduced drastically with the application of EM i.e. from 67.0 to 5.0 dSm<sup>-1</sup>.

##### **4.3.1.3 SAR**

Sodium adsorption ratio was 10.7 in case of EM soil while it was 79.0 in the original soil.

##### **4.3.1.4 Tube-well water**

Tube-well water was used for irrigation. Its composition was pH 8.1, EC x 10<sup>6</sup> 1446, CO<sub>3</sub> nil, HCO<sub>3</sub> 9.16 meq/l, Na 11.62 meq/l, Ca + Mg 2.84 meq/l, SAR 9.8 and RSC 6.32.

##### **4.3.1.5 FYM and Bokashi**

Animal's dung + urine (cow, bull, buffalo, goat, sheep and poultry manure) contain on an average N 3.62%, P<sub>2</sub>O<sub>5</sub> 1.33% and K<sub>2</sub>O 2.67%. Bokashi had pH 4.6, N 1.8%, P 210 ppm, K 960 meq/l, Mg 40 meq/l, Ca 72 meq/l, S 251 ppm, Cu 10 ppm, Zn 55 ppm, Mn 80 ppm, B 41 ppm, Fe 45 ppm and organic mater 84.54%.

#### **4.3.2 Determination of micronutrients (trace elements), heavy metals and Na in the CR-soil and EM-soil.**

The plants take thirteen mineral elements from the soil. Six elements {N, P, S (anion) and K, Ca, Mg (cation)} are classified as macronutrients, and 7 {Cl, B, Mo (anion) and Fe, Mn, Zn, Cu (cation)} are termed as micronutrients. This classification has been made according to the quantity of the elements taken by the plants. According to the chemical characters Fe, Mn, Zn, Cu, Mo are heavy metals. The heavy metals were determined to see the effect of EM on

the bio remediation of these metals as the soil on which the experiment was carried out contained some quantity of waste material of Himont Chemicals Pharmaceuticals.

Heavy metals and trace elements were determined with two methods i.e. 0.1 N-HCl leaching and inductively coupled plasma mass spectrometry (ICP-MS) and the results are given in table-2. The results of the two methods are almost identical. The perusal of the data indicates that the EM treated soil have less contents indicating that the EM with all its products have reduced the elements in the soil. The effect on the reduction of Na 49.2% and Cl 55.7% is specifically to be noted indicating that NaCl has been eliminated up to 50%. Al, Cr, Cd, and Fe have been reduced by 36%, 25%, 43%, and 45 % respectively. Boron has been reduced by 50%.

**TABLE – 1: Chemical analyses of original, CR- and EM Soil.**

Parameters	original soil*	CR soil**	EM soil**
pH	9.8	8.8	6.8
ECe dSm <sup>-1</sup>	67.0	53.0	5.0
OM %	0.41	0.48	2.10
Na meq/l	485	335	25
Ca+Mg meq/l	75	165	11
N %	0.02	0.02	0.45
Zn mg/kg	0.65	0.97	30.6
Cu mg/kg	0.3	1.01	1.58
Fe mg/kg	6.4	19.1	22.5
Mn mg/kg	9.3	10.0	12.5
SAR	79.1	36.9	10.7
Texture	loam	loam	loam

\* samples taken during first week of april 2000

\*\* samples taken during last week of october 2000 at the maturity of the rice crop.

**TABLE-2: Contents of trace elements, heavy metals and Na in the original and EM treated soil**

<b>Name of the elements</b>	<b>Methods</b>	<b>Original untreated soil (ppb)</b>	<b>EM treated Soil (ppb)</b>
Na	0.1 N HCl leaching	3200	
	ICP-MS	2900	1600
Cl	0.1 N HCl leaching	5560	2800
	ICP-MS	5500	2100
Al	0.1 N HCl leaching	130	95
	ICP-MS	130	70
Cr	0.1 N HCl leaching	25	26
	ICP-MS	35	19
Cd	0.1 N HCl leaching	35	18
	ICP-MS	28	18
Fe	0.1 N HCl leaching	520	300
	ICP-MS	520	270
B	0.1 N HCl leaching	2000	1000
	ICP-MS	1900	980

#### **4.3.3 Detection of anions, cations, and C & O in CR-soil and EM-soil.**

Anions were determined with neutral salt leaching method using leaching solutions M NaOH and 0.01 M Na<sub>2</sub> HPO<sub>4</sub>. The measured values were expressed in CgKg<sup>-1</sup>. Five typical kinds of negative ions that were found in the soil solution were Cl, SO<sub>4</sub>, HCO<sub>3</sub>, H<sub>2</sub>PO<sub>4</sub> and NO<sub>3</sub>. The measured value for the CR-soil and EM treated soil was 80 CgKg<sup>-1</sup> and 210 CgKg<sup>-1</sup> respectively indicating that eliminations / removal effect was higher (2.625 times) in EM treated soil as compared to CR-soil.

The cations were determined with surface scanning of the soil (XPS-X ray photo electron spectrometer) with which quantitative analysis and chemical bonding phase analysis of the specimen were conducted. As a result of wide scan Na, Fe, Ca, Si, Al, C and O were detected. The results in atomic % for CR-soil and EM treated soil are given in table –3. The perusal of the data indicates that Na, Ca, Fe is removed / reduced more in the EM treated soil compared to CR-soil. Both the cations and anions were removed more in EM treated soil as compared to the CR-soil. The presence of C & O in greater quantities in EM treated soil as compared to CR-soil (Table –3) suggests that the addition of materials (FYM, PM- compost, Bokashi) to the original saline-alkali soil for reclamation served as food to Effective Microorganisms contained in the EM and with that biomass and bacterial population increased tremendously and they secreted beneficial substances such as vitamins, organic acids, chelated minerals and anti oxidants into the soil solution.

#### **4.3.4 Determination of Enzymes in CR-soil and EM-soil.**

Enzymes activity in the CR-soil and EM-soil was examined. The enzymes extract in the respective soil was extracted with phosphoric acid buffer solution and the enzymes were estimated by an oxygen electro method. Enzymes activity was the least (on an average 229 U/L) in the CR-soil as compared to 2583 U/L on an average in the EM-soil (table 4).

#### **4.3.5 Determination of bacterial population in the CR-soil and EM-soil.**

The bacterial population in the CR-soil and EM-soil was determined by “YG-culture-media method” and bacteriological analysis by “dilution incubation of bacteria” (table –5 and fig –2 & 3). The data in table –5 indicate that the bacteria in the EM-soil are abundant than in the CR-soil, on an average the bacteria are more by  $5.3 \times 10^4$ . The bacteriological analysis (fig 2 & 3) showed that in EM-soil small colonies were of lactic acid bacteria as confirmed by the formation of air bubbles with the drops of hydrogen peroxide solution, and larger gray colonies were of bacillus group as presumed from the observations made on characteristics of colonies and identification of colors. The identification of various types of bacteria was made by ribo-printer system applying molecular biology technology and the bacteria identified are tabulated in table –6.

**Table –3 Detection of cations and C & O in atomic % in the CR-soil and EM-soil.**

Type of Soil	Na	Fe	Ca	Si	Al	C	O
CR-soil	1.0	0.1	0.1	14.6	15.6	10.5	48.0
EM-soil	2.5	1.7	5.5	14.2	16.6	17.0	56.8

**Table –4. Enzymes activity (U/L) in the CR-soil and EM-soil.**

Type of soil	1 <sup>st</sup> reading	2 <sup>nd</sup> reading	3 <sup>rd</sup> reading	Average
CR-soil	230	229	228	229
EM-soil	2643	2650	2456	2583

**Table –5. Measurement of bacteria in 10YG culture media.**

Type of soil	1 <sup>st</sup> reading	2 <sup>nd</sup> reading	3 <sup>rd</sup> reading	Average
CR-soil	16 x 10 <sup>4</sup>	12 x 10 <sup>4</sup>	12 x 10 <sup>4</sup>	13.3 x 10 <sup>4</sup>
EM- soil	20 x 10 <sup>4</sup>	18 x 10 <sup>4</sup>	18 x 10 <sup>4</sup>	18.6 x 10 <sup>4</sup>

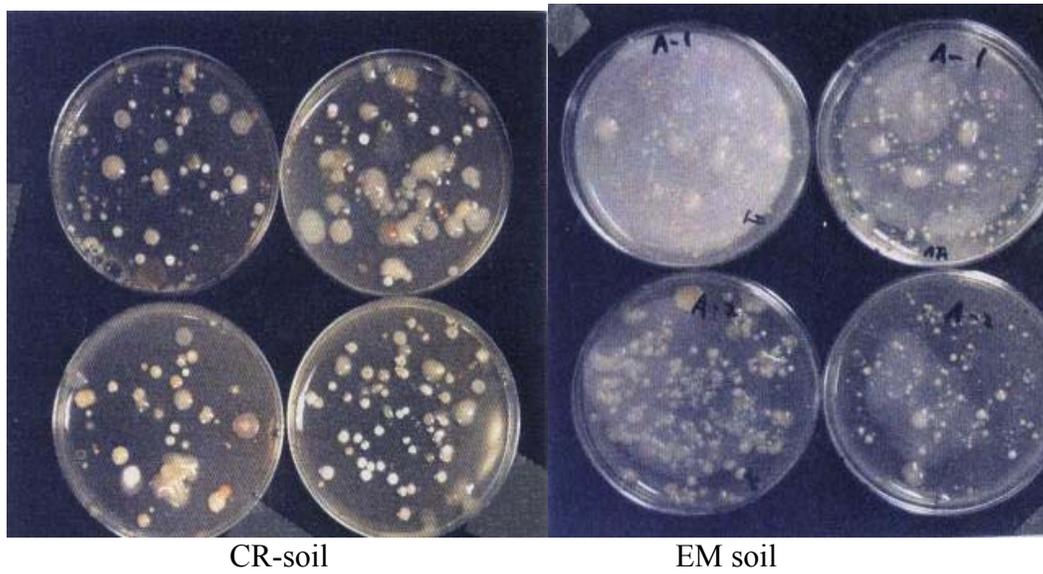
**Table –6. Types of bacteria in CR-soil and EM-soil**

Sr. No	Salt affected soil (control)	Sr. No	EM treated soil
1	Bacillus-sp	1	Azotobacter-sp
2	Entrobacter-sp	2	Bacillus -sp1
3	E. coli group	3	Bacillus-subtile
4	Fungi	4	Clostridium-treponema
5	Pseudomonas-sp	5	Corynebacterium-sp
6	Streptococcus-sp	6	Furabacterum
7	Serratia-sp	7	Gluconobacter-sp
		8	Lactobacillus- cassei
		9	Lactobacillus-sake
		10	Lactobacillus-sp
		11	Lactobacillus-sp1
		12	Lactobacillus-sp2
		13	Micrococcus-sp
		14	Micrococcus-sp1
		15	Micrococcus-sp2
		16	Pseudomonas- aeruginosa
		17	Pseudomonas- fluorescens
		18	Pseudomonas- putida
		19	Pseudomonas- Q1
		20	Pseudomonas- type –1
		21	Pseudomonas- type –2
		22	Pseudomonas-sp
		23	Rhodobacter-capsulatus
		24	Rhodoseubodomonas-sp
		25	Rhodospirillum-sp
		26	Streptococcus-sp
		27	Treponema-sp

**Fig -2. Dilution incubation of CR-soil and EM-soil**



**Fig -3. Isolation of colony of bacteria in CR-soil and EM-soil**



#### 4.3.6 Determination of chemical bonding of elements especially Si & Al

The photoelectrons energy emitted from a very shallow depth ( $10^{-9}$  mm) of soil is dependent on the peculiar bonding of elements. This can be measured by XPS (X-ray Photoelectron Spectrometry) techniques. Si & Al were observed at each waves-peek. The values for  $Al_2O_3$  and  $SiO_2$ , and Al-O-Si and Si-O-Al are given in table –7.

The study on C-bonding revealed that C is better bonded with H & O in the EM treated soil than in the CR-soil. This means that the absorption and elimination of salts in the CR lands is easy by using EM Technology in which abundant organic matter is used.

The perusal of the data shows that the concentration of  $Al_2O_3$  and  $SiO_2$  in CR-salt is abundant than in the EM-soil. On the other hand the Al-O-Si and Si-O-Al is more in the EM treated soil. The presence of oxides of Al & Si is considered to be not favorable for bacterial nourishment and growth, while complexes / chelates formed by Al & Si are not harmful to the microorganisms. More complexes were formed in EM treated soil as compared to the CR-soil (table –8). The formation of complexes / chelates helps to increase aggregation of soil particles and enhance the soil structures.

**Table –7: Elements waves-peek separation analysis**

##### i) Al2p

Peak position (eV)		Intensity (CPS)		Belonging to	Persistence (%)	
CR-soil	EM-soil	CR-soil	EM-soil		CR-soil	EM-soil
75.64	7528	418.1	419.9	Al (OH)	24.4	25.7
74.95	74.69	865.8	595.9	$Al_2O_3$	50.5	36.4
74.10	73.97	358.6	507.9	Al-O-Si	20.9	31.0

##### ii) Si2p

Peak position (eV)		Intensity (CPS)		Belonging to	Persistence (%)	
CR-soil	EM-soil	CR-soil	EM-soil		CR-soil	EM-soil
103.60	103.60	1346.3	380.6	$SiO_2$	49.8	16.7
102.85	102.85	1110.7	1482.6	Si-O-Al	36.4	65.8
101.81	101.79	1521.8	151.8	Si-O-Na	31.0	12.8
100.50	100.60	113.5	94.1	SiC	6.9	4.4

**Table –8: Complexes / chelates formed in CR-soil and EM-soil**

<b>CR-soil</b>	<b>EM-soil</b>
SiO <sub>2</sub>	KaSi <sub>2</sub> O <sub>8</sub>
(NaK) (Si <sub>2</sub> Al)O <sub>8</sub>	r-Al <sub>2</sub> O <sub>3</sub>
KaSiO <sub>3</sub> O <sub>8</sub>	PbTiO <sub>3</sub>
KaSiO <sub>3</sub> O <sub>8</sub>	(SiO <sub>2</sub> )X
(Ca.Na) (Si.Al) <sub>4</sub> O <sub>8</sub>	C <sub>4</sub> .4H <sub>12</sub> .85Al <sub>2</sub> Nil.150 <sub>8</sub> .
(NaK) (Si.Al) <sub>4</sub> O <sub>8</sub>	(Hoshimura et al.1999
(Al <sub>2</sub> O <sub>3</sub> )	05P1.80.22H <sub>2</sub> O
(Ca.Na) (Al.Si) <sub>2</sub> SiO <sub>8</sub> C	55C(C4H9)2NH
SiO <sub>2</sub> X	0.3(NH <sub>4</sub> )20.Al <sub>2</sub> O <sub>3</sub>
	0.95P <sub>2</sub> O <sub>8</sub> .O.20.Al <sub>2</sub> O <sub>3</sub>
	SiO <sub>2</sub>
	KaSi <sub>3</sub> O <sub>8</sub>
	KaSi <sub>3</sub> O <sub>8</sub>
	(Fe <sub>2</sub> O <sub>3</sub> )100M
	(NaK)AlSi <sub>3</sub> O <sub>4</sub> .1/2(NaK) <sub>3</sub> O.
	Al <sub>2</sub> O <sub>3</sub> .6SiO <sub>2</sub>
	NaO.61KO.39AlSi <sub>3</sub> O <sub>8</sub>
	(Na.Ca)Al.Si) <sub>4</sub> O <sub>8</sub>
	Ca7Na <sub>3</sub> Al <sub>1</sub> .7Si <sub>2</sub> .3O <sub>8</sub>
	Al <sub>2</sub> O <sub>3</sub>
	1/2(N,K) <sub>2</sub> O.Al <sub>2</sub> O <sub>3</sub> .6SiO <sub>2</sub>
	Ca.7Na.3Al.Si <sub>2</sub> .3O <sub>8</sub>

#### 4.3.7 Determination of organic acids in the CR-soil and EM-soil

The organic acids from the CR-soil and EM-soil were determined using solvent-extraction liquid with a high-speed liquid chromatograph (table –9).

The perusal of the data shows that the acetic acid and citric acid are abundant in the EM-soil while it could not be detected in the CR-soil. The lactic acid is more in the EM-soil (65.2 %) as compared to the CR-soil where it is only 0.39 %. The decay related butyric acid was not detected in the EM-soil while it was 0.45 % in the CR-soil.

The organic acid are said to couple soil particles to form aggregates especially the citric acid acts like a paste for joining the soil particles together. This phenomenon suggests that aggregates structure is formed in the EM-soil as compare to the CR-soil. The same was confirmed with surface scanning of the soil using an electron microscope (Fig –3 & Fig –4).

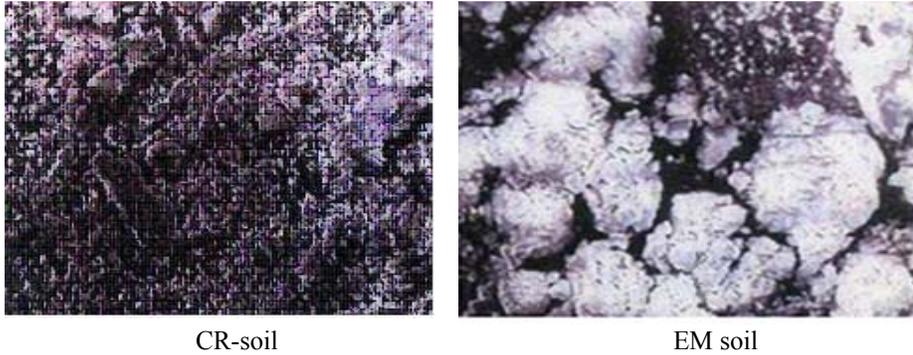
**Table –9: Organic acids in the CR-soil and in the EM-soil**

Organic acids (%)	1 <sup>st</sup> rdg*	2 <sup>nd</sup> rdg	3 <sup>rd</sup> rdg	4 <sup>th</sup> rdg	5 <sup>th</sup> rdg	Average (%)	
Acetic acid	EM soil	0.75	0.87	0.79	0.80	0.89	0.82
	Control	ND**	ND	ND	ND	ND	-
Butyric acid	EM Soil	ND	ND	ND	ND	ND	-
	Control	0.36	0.56	0.50	0.40	0.46	0.45
Lactic acid	EM Soil	0.60	1.08	0.96	1.39	1.56	1.12
	Control	0.45	0.70	0.54	0.14	0.14	0.39
Citric acid	EM Soil	1.70	1.75	1.75	1.90	1.78	1.78
	Control	ND	ND	ND	ND	ND	-

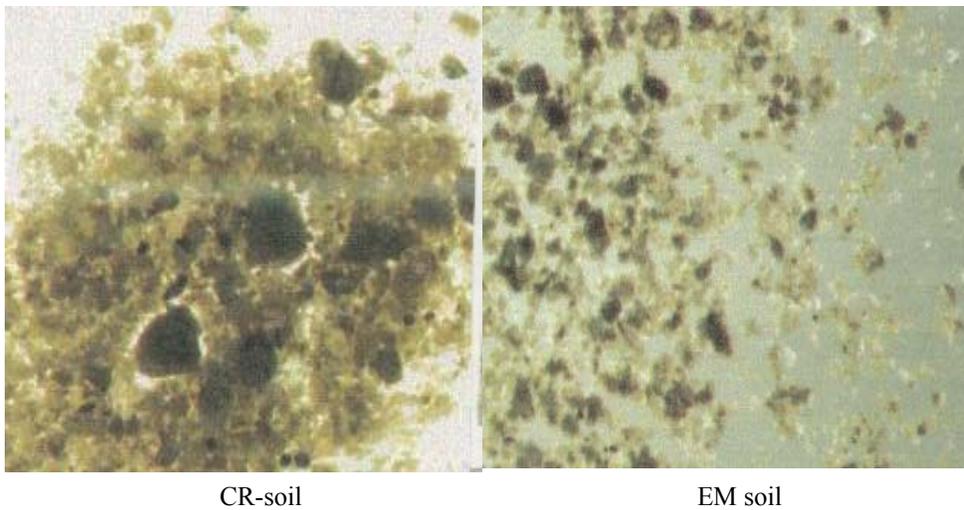
• rdg = reading

\*\* ND = not detected

**Fig -3. The image showing the aggregate structure in EM-soil**



**Fig -4. Presence of crystals of salt surrounded by black decayed substances in the CR-soil.**



#### 4.3.8 Crop performance

Rice crop was grown in the CR-soil and EM-soil. Observations made on mortality showed that it was 30.9 % more in the CR-soil as compare to EM-soil. This affected also the number of tillers per plant, which were 10 in case of EM-soil and 6 in case of CR-soil. There were on an average 55 – 64 grains more per rice plant comprising of number of tillers in the EM-soil as compare to CR-soil. The plants were on an average 10 –15 cm higher in the EM-soil than CR-soil. The crop was harvested during 10 – 20 November 2000. The yield per acre was 1490 Kg in the EM-soil while it was 1120 Kg in the CR-soil.

The contents of protein were 8.7 % in the EM-soil while it was 8.4 % in the CR-soil. Similarly the fats were 0.8 % in the EM soils and 0.7 % in the CR-soil. Carbohydrates were 77.5 % in the CR-soil while it was 77. % in the EM-soil. There was no difference in the contents of cruds fiber, which was 0.4 % in both the cases.

The occurring of diseases and attack of insects was more in the CR-soil than in the EM-soil. The regular spraying of EM extended diluted by 1000 times helped to 0control insects attack and occurring of diseases in the EM treated plots.

#### 5. Mechanism of EM (Effective Microorganisms)

The application of EM in all possible forms (soaking of seeds, 2.0 tons / acres composted FYM + PM, 100 Kg / acre Bokashi, EM irrigations and EM sprays) played a pivotal role in reclaiming saline-alkali land in the 1<sup>st</sup> year and consequently production of good yield of rice grain as compared to conventional method of reclamation, which generally takes about 2-3 years to fully reclaim and resuscitate the soil fauna and flora. In the presence of easily decomposable organic matter, the overwhelming majority of Effective Microorganisms i.e. phototrophic bacteria lactic acid bacteria and yeast being aerobic and anaerobic in nature, coexisted symbiotically well with other soil microorganisms.

The soil microorganisms act also as decomposers of organic matter particularly polysaccharides, lignin and chitin with the production of humus, initiators of C & N cycles and producers of antibiotics and killers of pathogens. The distribution and multiplication of microorganisms in soil is determined largely by the presence of food supply in the surface soil. They, therefore, occur in the greatest number in the upper soil horizon and had teeming mass of biological activity in the presence of optimum food, moisture and temperature. Generally bacteria are present in numbers of  $10^8$  to  $10^9$  organisms per gram of soil (300 to 3000 grams of biomass per  $m^3$  of soil), actinomycetes are  $10^7$  to  $10^8$  organisms per gram of soil (300 to 3000 grams of biomass per  $m^3$  of soil) and fungi are  $10^5$  to  $10^6$  propagules per gram of soil [600 to 10 000 grams of biomass per  $m^3$  of soil [Cinklin, Jr. A. R. *soil microorganisms contaminated soil sediment and water. The magazine environmental assessment and remediation, Jan / Feb., 2002*]. The number of groups of microorganisms that commonly occur in top 0-15cm i.e. per hectare-furrow slice may be for bacteria  $10^{17}$ - $10^{18}$  with fresh biomass 450-4500 Kg, for

actinomycetes  $10^{16}$ - $10^{17}$  with fresh biomass 450-4500 Kg, for fungi  $10^{14}$ - $10^{15}$  with fresh biomass 112-1120 Kg, for algae  $10^{13}$ - $10^{14}$  with fresh biomass 56-500 Kg and for protozoa  $10^{13}$ - $10^{14}$  with fresh biomass 17-170 Kg. The microflora (bacteria, actinomycetes, fungi and algae together form 2076 to 20760 Kg fresh biomass and 415-5190 Kg dry biomass (20-25% of fresh biomass) per HFS (*Brady 1994*). According to Alexander 1977 (*introduction to soil microbiology. John Willey & Sons. NY. 467p.*) the microbial number in the top soil (3-8 cm) may be 12 million/g, comprising live biomass of bacteria 100-4000 Kg/ha; while double of this biomass for all the microbes (*bacteria, actinomycetes, fungi and algae*) forming 0.02-0.8% of total soil biomass [*referred by Sikandar, A., nuclear institute for agriculture and biology, Faisalabad, Pakistan: Effect of organic and inorganic Fertilizers on the Dynamics of Soil Microorganisms, Biomass, Composition and Activity.*]

The photosynthetic bacteria (*rhodospseudomonas spp*) synthesize useful substances such as amino acid, nucleic acid, bioactive substances (vitamins, enzymes and hormones) and sugar. These are released into the soil solution. The presence of amino acids (a nitrogenous compound) increase the population of Vesicular Arbuscular micorrhiza in the rhizosphere, which in turn enhance the solubility of phosphates in the soil and can coexist with *Azotobacter* and *Rhizobium*, thus, increasing the capacity of plants to fix atmospheric N. the photosynthetic bacteria is considered the pivot of EM Technology [*Higa, Teruo 1993*].

Lactic acid bacteria (*lactobacillus spp*) produce lactic acid using sugars and carbohydrates. Lactic acid is a strong sterilizing compound, suppresses harmful microorganisms (*Fusarium*) thus reducing nematode population, enhances decomposition of organic matter, promotes fermentation and decomposition of material such as lignin and cellulose [*Higa, Teruo 1993*].

The yeast (*saccharomyces spp*) synthesizes anti microbial and other useful bioactive substances such as hormones and enzymes, which are useful substrates for effective microorganisms [*Higa, Teruo 1993*].

The fungi break down highly complex and resistant compounds such as cellulose, starch, gums and lignin [*Cinklin, Jr. A. R. 2002*].

Actinomycetes produce and release in the soil solution antibiotics such as streptomycin, actinomycin and neomycin, and are involved in the decomposition of complex organic compounds such as phospholipids [*Cinklin, Jr. A. R. 2002*].

Chemistry and biology in soil environment (solids, liquids & gases) are significantly different and more complex. Microorganisms produce a large variety of byproducts, some of which are quite surprising. N containing compounds are decomposed and ammonia ( $\text{NH}_3$ ) and ammonium ( $\text{NH}_4$ ) are released into the soil solution. This in turn is oxidized for energy by other bacteria producing nitrite and nitrate. The rate of nitrate production is faster than nitrite production. Some free-living and some symbiotic bacteria can take N from the air and combine it with

organic compound to produce amino acids. Humus is the material remaining after decomposition of organic matter. It is made of three components: fluvic acid, humic acid and humin. Its particles are colloidal in size. It has high sorptive capacity for water and organic compound as well as high cation exchange capacity (CEC). At basic pH, CEC increases, thus, both organic molecules and cations, but not anions, are attracted to humus [Cinklin, Jr. A. R. 2002].

The decomposition of organic matter means that a diversity of bio-and organic molecules (acid, alcohol, ether and aldehyde) are constantly being released into the soil solution. Under aerobic soil the decomposition of organic matter / molecules the main products are CO<sub>2</sub> and H<sub>2</sub>O, e.g. [Cinklin, Jr. A. R. 2002].

R-CH<sub>2</sub>CH<sub>3</sub> + O<sub>2</sub> aerobic microorganisms CO<sub>2</sub> + H<sub>2</sub>O + Energy + Mineral  
+ Humus

Under anaerobic soil the microorganisms break down organic (C- containing) compounds for energy and final products are:

R-CH<sub>2</sub>CH<sub>3</sub> + O<sub>2</sub> anaerobic microorganisms CH<sub>4</sub> + H<sub>2</sub>O + Energy  
+ Mineral + Humus

Methane is the simplest and reduced form of organic compounds in soil. It is considered the basic unit from which all organic molecules are built [Cinklin, Jr. A. R. 2002].

Summarizing it can be concluded that decomposition processes in soil release minerals, macro-and-micro nutrients (N, P, K, S, Ca, Mg, K, Fe, Mn, Zn, Cu, etc) and other by products such as amino acid, sugars, fatty acids, organic acids, chelates, NH<sub>3</sub>, NH<sub>4</sub>, NO<sub>3</sub>, organic molecules (acid, alcohol, ether, aldehyde), humus (fluvic acid, humic acid and humin), vitamins, hormones, enzymes and antibiotic etc.

The results obtained confirm the multiplication of useful bacterial population, the production of enzymes and their activity, production of organic acids (lactic acid, acetic acid, citric acid), formation of aggregates and chelates, removal of NaCl and elimination of exchangeable Na<sup>+</sup> and higher germination, less attack of insects, minimum occurrence of diseases and higher yield of rice grain.

Now the question arises how EM has ameliorated saline-alkali sodic land and what is the possible mechanism of Effective Microorganisms to reclaim such lands. According to Agriculture Handbook No. 60, United States Department of Agriculture a saline-Alkali Soils are characterized by their appreciable contents of soluble salts (ECe > 4 mmhos/ cm) and exchangeable sodium percentage (> 15). The pH may vary considerably. Cl & SO<sub>4</sub> are the principal soluble anions, HCO<sub>3</sub> content is relatively low, and CO<sub>3</sub> is absent. The soluble Na contents exceed those of Ca + Mg. This means that the problematic zone was the rhizosphere,

being saline-alkali in nature and has higher contents of Na in the soil solution and exchangeable Na > 15. This is of major concern. If these are controlled and brought to acceptable level, then the soils are said to be reclaimed and agricultural crop can be grown. Now coming to the level of reclamation achieved, which is reflected by the reduction in pH from 9.8 to 6.8, ECe from 67 to 5 dSm<sup>-1</sup> and SAR from 79 to 11. The formation of aggregate structure by the release of organic acid and humus by the effective microorganisms in combination with organic manure and Bokashi improved the structure of the upper soil and with that the permeability of the rhizosphere. The tremendous activity of thousand billions of Effective Microorganisms in the presence of composted FYM + PM and Bokashi, released many byproducts inclusive of NH<sub>4</sub><sup>+</sup> and Ca<sup>++</sup> ions (Bokashi contained 72 meq/L or 1442 ppm Ca) into the soil solution. These exchanged the exchangeable Na from the clay complex and Na became a part of the soil solution. Na present in the solution formed NaCl and Na<sub>2</sub>SO<sub>4</sub>. These being soluble in water, leached from the upper most soil horizon (plough layer) to the moderate permeable loamy lower layers of the soil profile. This is the possible mechanism of EM in soil reclamation.

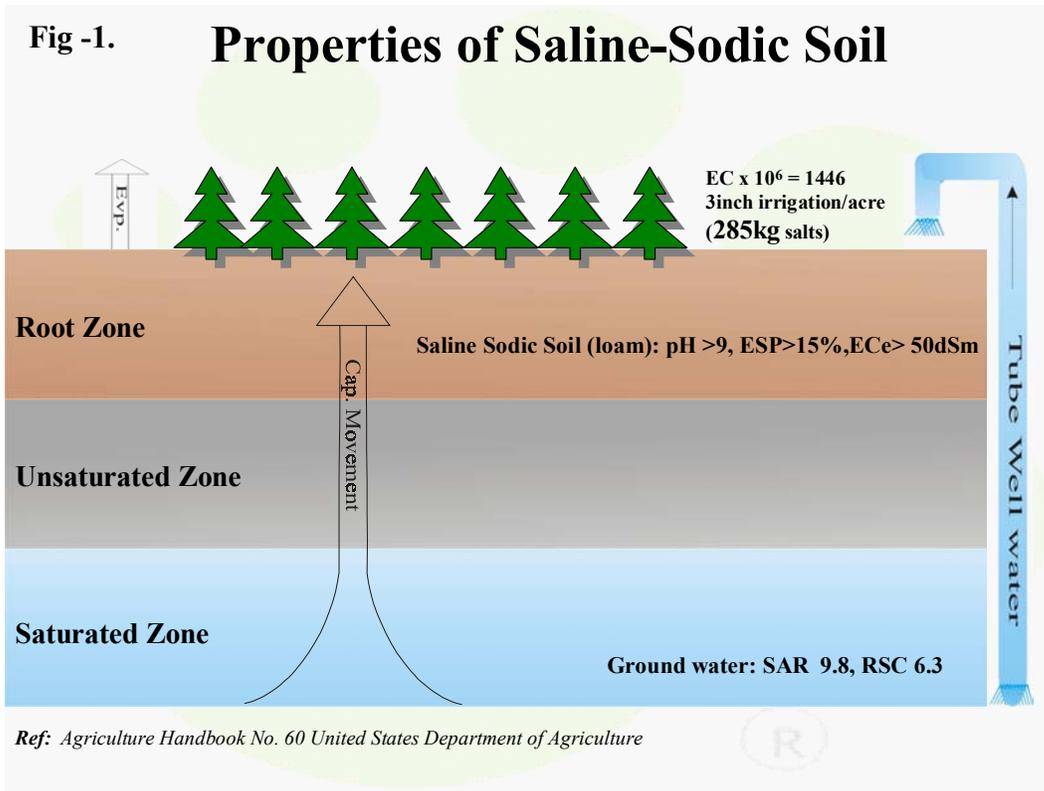
It has been tried to represent the properties of saline-alkali soils, reclamation practices and mechanism of reclamation with canal water, sulfuric acid, gypsum and EM in figures. For this purpose four figures have been prepared for better understanding at a glance and the same are given as Fig -1, Fig -2, Fig -3 & Fig -4.

## 6. Conclusion

From the results of the field experiment of “reclamation of saline-alkali soil”, it can be concluded that the reclamation and amelioration of saline-alkali soil can be achieved most effectively with the application of EM Technology, which comprises of soaking of seeds in EM solutions, applications as EM irrigations and EM sprays to the crop, addition of farmyard manure + poultry manure composted with EM solution and Bokashi (rice bran fermented with EM solution). The overwhelming majority of Effective Microorganisms thousand billions in number in the presence of easily decomposable organic matter co-exist symbiotically with other bacteria, fungi, actinomycetes and soil fauna and flora. They synthesize and release useful substances organic acids (amino acids, nucleic acids, citric acids, acetic acids, lactic acids), alcohols, ethers, aldehydes, bio active substances (vitamins, enzymes, hormones), sugars, polysaccharides, and antibiotics (streptomycin, actinomycin, neomycin). They enhance solubility of phosphates, fix atmospheric N forming NH<sub>4</sub> and NO<sub>3</sub>, break down highly complex and resistant compounds (cellulose, starch, gums, lignins). They produce humus (fluvic acid, humic acid, humic) and release macro- and micro nutrients (N, P, K, S, Ca, Mg, Fe, Mn, Zn, Cu, etc) and other products like fatty acids, chelates etc into the soil solution. The NH<sub>4</sub> and Ca replace the Na<sup>+</sup> from the clay complex. Na<sup>+</sup> becomes the part of the soil solution and form NaCl and Na<sub>2</sub>SO<sub>4</sub> in the soil solution and these salts leach down to the lower layers of the soil profile, making the upper zone of the rhizosphere free from harmful salts. This part of the root zone becomes biologically extremely active releasing all types of essential nutrients to the roots for uptake by the plants. The saline-sodic

soil is not only reclaimed in the 1<sup>st</sup> year without using any soil amendment such as gypsum or sulphuric acid etc but it gives also good production. In short EM Technology is effective, easy to prepare and use and leaves behind enhanced bacteria population increasing soil fertility for all times to come.

**Fig -1. Properties of Saline-Sodic Soil**



**Fig -2. Saline-Sodic Soil Reclamation Practices**

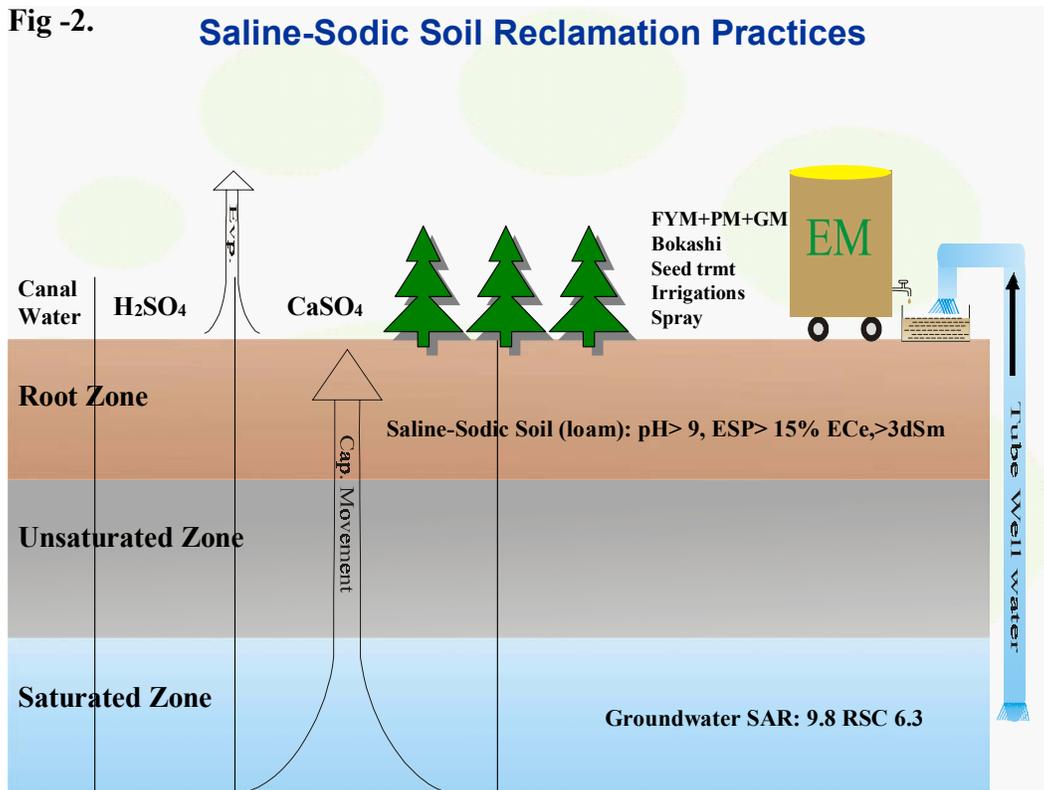


Fig -3.

## Saline-Sodic Soil Reclamation Practices

H <sub>2</sub> O	H <sub>2</sub> SO <sub>4</sub>	CaSO <sub>4</sub>	FYM+PM+GM Bokashi Seed trnt Irrigation Spray
<ul style="list-style-type: none"> <li>-requirement of 3-4 ft/acre canal water for irrigation</li> <li>- improvement of land with special management practices</li> <li>- retarded germination and emergence of seedlings</li> <li>- rowing of salt tolerant crops (rice, sugar beet, spinach, clover, barley, wheat, sugarcane) necessity of inorganic/organic fertilizers</li> <li>(N 100, P 22 &amp; K 50 kg/ha)</li> <li>{12,192m<sup>3</sup> water}</li> <li>- reclamation period 3-5 years</li> </ul>	<ul style="list-style-type: none"> <li>-50-200 t/acre depending upon the alkalinity</li> <li>-transportation difficult</li> <li>-application cumbersome, danger of burning, hence not popular</li> <li>-presence of CaCO<sub>3</sub> in the soil essential</li> <li>-growing of salt tolerant crops</li> <li>- application of inorganic/organic fertilizers</li> <li>-(N 100, P 22 &amp; K 50Kg/ha)</li> <li>-reclamation period 2-3 years</li> </ul>	<ul style="list-style-type: none"> <li>-requirement of 6-10 tons/acre-ft gypsum depending upon the CEC, exchangeable Na and ESP</li> <li>- solubility of gypsum in water at ordinary temperature about 0.25%</li> <li>- growing of salt tolerant crops</li> <li>- application of inorganic/organic fertilizers</li> <li>-(N 100, P 22 &amp; K 50Kg/ha)</li> <li>-reclamation period 2-3 years</li> </ul>	<ul style="list-style-type: none"> <li>-practicing GM beneficial to improve physical properties of soils</li> <li>-Requirement of OM (2 tons/acre)</li> <li>(FYM, PM: N 178, P 28 &amp; K 110 kg/ha, available after mineralization)</li> <li>-EM solutions (40 lit/acre, 1:20 extended)</li> <li>-Bokashi (100 kg/acre)</li> <li>- Spraying with diluted EM-extended</li> <li>- Preparation, handling and application easy and safe, and leaves behind no harmful/residual effects</li> <li>- No need of inorganic fertilizers</li> <li>{12,192m<sup>3</sup> water}</li> <li>- reclamation period 1 year, cost effective</li> </ul> <p>FYM+PM: pH 6.5, N 3.6%, P<sub>2</sub>O<sub>5</sub> 1.3%, K<sub>2</sub>O 2.7%                      Bokashi: pH 5.6, N 1.8%, K 960, Mg 40 &amp; Ca 72meq/l, OM 85%</p> <p><i>Ref: Hussain, T. et al: Brackish ground water use technology in salt affected soil through soil fertility management, Journal of drainage and reclamation, July-Dec, 1991, Vol. 3, No.2</i></p>

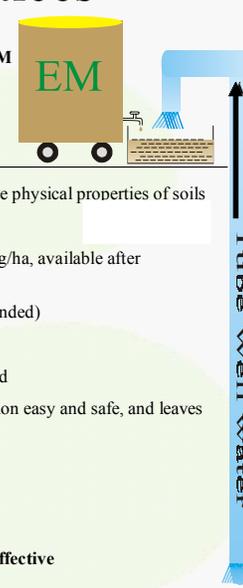


Fig -4.

## Mechanism of Reclamation of Saline-Sodic Soil

H <sub>2</sub> O	H <sub>2</sub> SO <sub>4</sub>	CaSO <sub>4</sub>	FYM+PM+GM Bokashi Seed trnt Irrigation Spray
<p style="writing-mode: vertical-rl; transform: rotate(180deg);">Canal water leaching</p>	$\begin{matrix} \text{H}_2\text{SO}_4 + \text{CaCO}_3 \\ \uparrow \\ 2\text{NaX} + \text{CaSO}_4 \end{matrix}$	$\begin{matrix} \text{CaX} + \text{Na}_2\text{SO}_4 \\ \uparrow \\ 2\text{NaX} + \text{CaSO}_4 \end{matrix}$	<ul style="list-style-type: none"> <li>- increase in microbial population, which helps to maintain fertility in the rhizosphere</li> <li>- decomposition of organic matter</li> <li>- release of energy and organic acids: amino, lactic, acetic, citric and butyric</li> <li>- availability of macro and micro nutrients: NPK, Ca, Mg, K, Cl, B, Mo, Fe, Mn, Zn, Cu</li> <li>- Improvement of physical properties: water holding capacity, aggregation of soil particles &amp; porosity</li> <li>- <b>exchange of ions:</b> application of compost of FYM + PM, GM/Green leaves, Bokashi &amp; EM Solutions having pH 6.5, 5.0, 5.6 &amp; 3.5 respectively decreases pH from alkalinity to neutrality in the reclaimed soil. The organic acids in the presence of large quantity of irrigation water (acidic medium) attain 100% disassociation (considerably weaker than N/10) producing not only large quantity of H-ions but also releasing Ca from the CaCO<sub>3</sub> of the soil and Bokashi, thus H and Ca ions replace Na from the clay complex forming leach able Na<sub>2</sub>SO<sub>4</sub>.</li> <li>Photosynthetic bacteria has the characteristics to accept Cl from NaCl and produce protein. Yeast contains Apo-protein-A &amp; B which can convert NaCl into protein and chelates. Salt tolerant bacteria has the characteristics to de-ionize NaCl in the soil.</li> </ul> <p><i>Ref: -Biochemistry in Agricultural Sciences Vol. II by S. S. Bhatia, pp 4</i>                      -Alfred R. Cunklin, Jr: Soil microorganisms. Contaminated Soil Sediment &amp; Water, Jan/Feb 2002, pp 12-14</p>

