### Remediation and Preservation of Natural Ecosystems through Application of Effective Microorganisms (EM)

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Abstract: For the last several decades, humankind has been trying to have an advanced civilization spending much energy made by natural resources. Increased world population has created great amounts of waste and environmental pollution, especially with dioxin. Modern conventional agriculture also has many problems, led by utilization of agrochemicals. The excess usage of pesticides can be the cause of poor soil fertility and polluted agricultural products. The pesticide can break down not only human health, but, also environment. The application of microbes, especially Effective Microorganisms (EM), for the control of pollution of the environment is getting popular. EM is a microbial mixture solution and it includes only beneficial aerobic and anaerobic microbes. Therefore it is very safe to use EM on environmental improvement.

When EM was applied, the metabolic substances, such as antioxidants, organic acids, nucleic acids, enzymes and minerals, will work on harmful substances like dioxin to change into harmless character. EM can create considerable powers of self-purification in nature.

#### Introduction

Most of pesticides, which have been applied on agricultural fields, can be decomposed by many kinds of microbes, physiochemical or multiple action in soil such as hydrolysis, oxidation, reduction, isomerization, polymerization and condensation. SMSJ (1999). However, most living things in the soil, which have been exposed to pesticides, have resistant property to harmful chemical substances. Organic phosphorous insecticide - Fenitrothion (MEP), needed for killing insects which are a problem in fields used for agriculture and forestry was used to study decomposition using EM.

# Materials and Methods

This study was conducted to compare two types of soil in Taiyo-mura, Ibaragi Prefecture, Japan. One is conventional agricultural fields, which use MEP regularly, and the other fields have applied EM Bokashi, extended EM and pig manure, which are treated by EM for soil management for the last three years.

Physico-chemical property of both soils - such as soil aggregate, EC, pH were analyzed in this study. The impact of EM application on micro-flora and decomposed property of MEP in soil were investigated. When the microbes such as ray fungi, bacteria and fungi were selected and isolated from the soil, low concentrate general organic medium (diluted 20 times) was used.

#### Pot test

The decomposed property of MEP in soil was inspected by the following method- 50g of soil was taken as sample from the conventional agriculture field which has residual MEP ( $236\mu g/g$ ) and the field using EM Technology. Each sample was put into 100ml polypropylene bottles and added MEP (Takeda: 50% of MEP, including emulsifier and solvent) and the final MEP density was adjusted  $333\mu g/g$  respectively. Both samples, which were in slurry condition, were kept for 10 days, and then the remains of agrochemical in both samples were analyzed with GC and GC-Mass methods. The micro-flora also was inspected.

#### Separation and refining of soil sample

Around 120g of soil sample was passed through a 300-mesh filter to make the grain size of the soil uniform. Methanol was extracted from the samples using various solvents. Then the methanol was analyzed with HPLC - UV280mm and the chemical structure of the methanol was analyzed with GX400FT-NMR (Nihon Electronic) regarding H-NMR and C-NMR.

**Results** 

The results of field inspection can be seen in Figures 1 and 2.

Leucine, which originates in MEP, was found in the soil sample of conventional agriculture fields. Organic phosphorous compound was not detected in the soil sample of the field which applied EM, however, according to the chemical structure analysis, it was verified that phenol substances, which are a part of humus ingredients, were found in the soil sample. The analyses of soil sampling are shown in Figure 1 and 2.

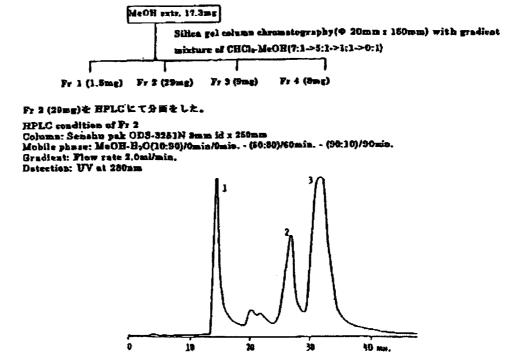


Figure 1. Conventional Agriculture Soil

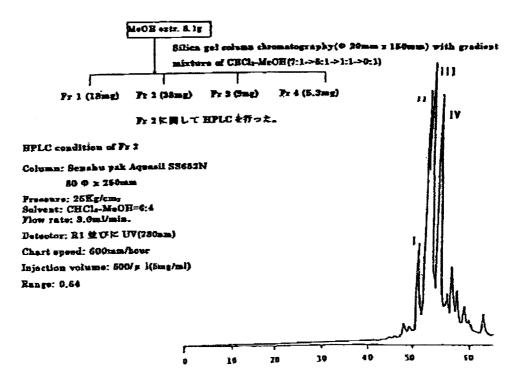


Figure 2. The Field using EM

When microbes decompose MEP, it is important to hydrolyze the phosphate and nitro group. Figure 3 shows the breakdown process and MEP is oxidized again to carbon dioxide.

The aggregate structure and micro-flora in the soil, which has used EM for the last three years, was developed progressively. There are many pore spaces in the soil. a variety of microbes such as fluorescence Pseudomonas and algae colonies on the agar media, especially, an increment number of ray fungi, which can produce various bioactive substances.

In contrast, the soil structure, which has applied pesticide or MEP, was undeveloped and the microbes, which have tolerance to pesticide, appeared on agar media. The variety of microbes was simple and the number was low.

This experiment shows that application of EM to the soil can suppress pesticide with decomposition process of harmful substances (Table 1).

Table 1. The Result of Decomposition Test on MEP

Sample	MEP Remains μg/g	Remains / MEP %
Non Organic Farm Soil	569.0	171
Organic EM Farm Soil	76.4	23
MEP Added (Blank)	333.0	100

In the control soil, which has used MEP for the last three years, any microbes were not able to appear on the media and the high density of MEP residues was detected at  $569\mu g/g$  (171%).

In the EM soil, which has applied EM for the last three years, the MEP residues were detected at  $76.4\mu g/g$  which decreased by 77%.

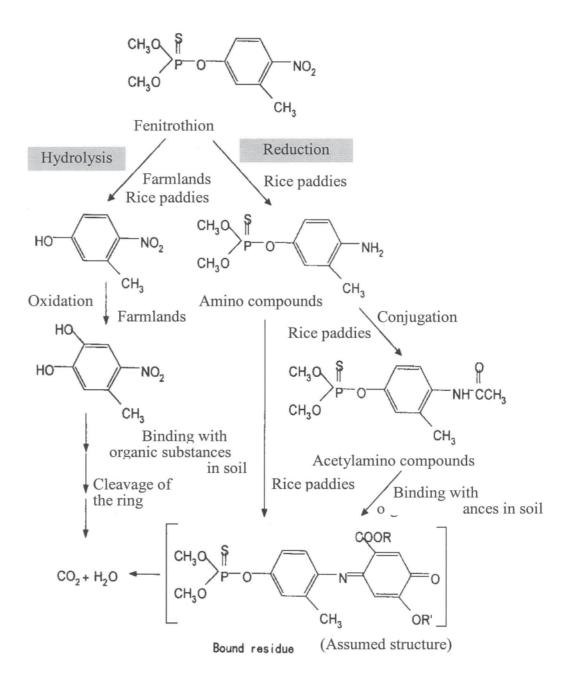


Figure 3. Decomposition of Organophosphorous Pesticide Fenitrothion in Soil (Nambu, 1990 modified)

#### A Study on Decomposition of Dioxin with EM

#### Introduction

Dioxin, which works as an environmental hormone, is causes detrimental effects to many plants and animals through the food chain, particularly the herbicide (CNP: Chrolonitrophen), which is applied to paddy and contains dioxin. Therefore the fields accumulated dioxin for a long term.

In this study, the decomposition capacity of dioxin in the soil was compared between the agricultural fields, which have applied EM as soil improver, and the soil from conventional agriculture method using agrochemicals. Both types of fields were around a garbage incineration factory. In addition, EM solution was added to both kinds of soils, which were in pots, and effectiveness of EM was studied on decomposition of dioxin and the micro-flora in the soil.

# Materials and Methods

#### Soil sampling

In 1999, the soil samples were gathered from five points in a plot, and mixed together as a sample.

#### Sample 1: Tonegawa-cho, Ryugasaki-shi, Ibaragi Prefecture, Japan

There are two sampling plots in this area. One was a control, which has been cultivated with conventional method using agrochemicals. Another plot was a paddy field, which has been applied 200 - 300kg of EM Bokashi per 0.1ha for two years. Both plots were within 2km in distance from an industrial waste incineration unit.

### Sample 2: Tokorozawa-shi, Saitama Prefecture, Japan

The soil as control was sampled in fields, which was using agrochemicals in Mt. Kunugi, which has several industrial waste incineration units. Also the soil sample was taken from the kitchen garden using kitchen wastes treated with EM in the Mt. Kunugi area.

#### Sample 3: Nose-cho, Osaka Prefecture, Japan

The soil as control was sampled in fields, which was using agrochemicals. Also the soil sample was taken from the field using EM Technology. Both fields were within 1km distance from an industrial waste incineration unit.

The character and the organisms-flora of the sampling soils were inspected and the decomposition of dioxin (CO-PCB) was analyzed with GC-HR-MS.

50g each of the control soils and the soils with treated EM were put into 500ml volume bottles. After adding 300ml of tap water, they were shaken well for 30 seconds. All bottles were kept near a window during the summer season in Japan, August through October. The room temperature was 24 - 36  $^{\rm OC}$  and organisms-flora were inspected with a microscope.

#### Pot Test

To 250g of the soil from paddy fields, which have used agrochemicals in Tonegawacho, Ryugasaki-shi, Ibaragi Prefecture in Japan, were added 2.5ml of EM (1% of the sample soil), 7.5g of EM Bokashi (3%), 50mg of EM-X ceramics. The soil in pots was kept in an incubator and the GC-HR-MS of dioxin was analyzed on 12th and 50th day.

## Results and Discussion

#### The soil from paddy fields applied with EM

- The soil aggregation is very fine so that the soil particles can precipitate quickly even after shaking for 30 seconds. The water was clean on second day (the control is on ninth day).
- Chlorella and water fleas appeared from the third day and they were growing in the suspension.
- Growth of green algae and filamentous algae were observed from the ninth day on the soil.
- · Diatoms were growing on the wall of the bottle from twelfth day.
- Many kinds of life such as bacteria, algae and protozoa were able to coexist in the bottle
- After three months, the living things lived with adding water. Even after one year
  passed from starting this observation, still the living things could live and keep the
  circulatory system among microbes, algae, protozoa and plant.

When water was added into the soil applied EM, first, bacteria increased in number, next, algae such as chlorella, diatom, spirulina and protozoa like ciliate, water fleas appeared. At the same time, biodiversity was investigated in the bottle. Once the biodiversity occurred, the flora of the living things in the bottle diversified and many kinds of enzymes were produced. The living things got the ability of decomposition or composition of chemical substances, which are hard to decompose.

Alternation of generations took place, however, the all living things never died so that the clearness of the water was not changed so much.

According to investigation of living things flora, particular kinds of plant, insect, fish, shellfish, amphibian and reptile were observed. It indicated that the ecosystem tended to recover.

#### The soil using chemical fertilizers and pesticides

Not only delay of propagation, also decrease or extinction of living things were investigated in the bottles. Once water fleas died, bacteria appeared and clearness of the water became low. The residual pesticides in the soil are the reason of the phenomenon and it might be thought that environmental pollution and excess use of agrochemicals reduce the number and variety of living things.

It was not possible to investigate algae on the soil, however, only surface of the water. The variety of living things was very few and growth process was slow. After three month, just one weed grew on the soil and algae floated on the surface of the water. Any other living things could not be observed with the naked eye and a loupe.

### A field test on decomposition of dioxin (Table 2)

In this test, the sample soil that was collected from the area within 2km from the waste incineration unit, was analyzed about dioxin and CO-PCB. The sample soils from paddy fields were investigated regarding organism-flora and compared between control, which applied agrochemicals, and the soil sample, which applied EM.

Table 2. The Level of Dioxin Contamination in Top Layer (0 - 5cm) around Incinerators

Test Area Soil Type	Ryugasaki-shi Paddy		Nose-cho Upland		Tokorozawa-shi Kitchen Garden	
	Cont.	EM	Cont.	ріани ЕМ	Cont.	EM
Dioxin						
pg-TEQ/g	110	7.6	74	14	19	12
		(93% less)		(81% less)		(37% less)
CO-PCB						
pg-TEQ/g	1.1	0.55		NT		NT
		(50% less)				

NT: Not Tested

### The result of analysis on dioxin and CO-PCB

Test 1. (Paddy fields: Ryugasaki-shi) The content of dioxin was 110pg-TEQ/g in the sample soil, which was using the conventional agriculture method. On the other hand, the sample soil from the paddy fields, which applied enough EM repeatedly, included 7.6pg-TEQ/g (93%). The content of CO-PCB was 0.55pg-TEQ/g (50%), which is lower than control.

Test-2. (Vegetable fields: Nose-cho) the content of dioxin was 74pg-TEQ/g as control and the sample from vegetable fields was 14pg-TEQ/g (81%).

Test 3. (Kitchen gardens: Tokorozawa-shi) The total content of dioxin in the soil of the kitchen gardens, which applied just few EM, reduced to 12pg-TEQ/g (37%)

The above three tests indicate that the application of EM can reduce the total content of dioxin in upland, paddy and even kitchen gardens fields.

#### The result of pot test - decomposition of Dioxin in paddy field

The decomposition of dioxin was investigated on the twelfth day and fiftieth day after treatment. Table 3 shows that the amount of dioxin decreased by 24% on the fiftieth day.

Table 3. Dioxin Content before and after EM Treatment in Pot Test

	Before EM Treatment	After EM	I Treatment
		12 days	50 days
Dioxin Pg-TEQ/g	170	150	130
		(12% less)	(24% less)

Harmful substances can be changed to harmless substances

Over 40 kinds of minerals can be found in EM solution. It included titanium oxide, which is a kind of photocatalyst and exist in nature. When harmful substances spread in the air, those minerals and ultraviolet rays oxidize and decompose the harmful substances. It is the process of making harmful substances harmless.

#### Discussion

Soil have accumulated the pollution of the environment during the last few decades, especially, dioxin in the soil and organochlorine pesticides can be adsorbed to soil particles and finally flow into rivers, lakes and sea. The living things in the sea take the harmful substances and it will be concentrated biologically in their bodies. Birds and animals, which had the polluted material, got very serious problems. At present, more than 100 kinds of chemical substances are detected in the blood of human beings and 30 - 40% of neonates have allergies.

All living things on the earth have a lot of common genes, therefore, human beings are not able to exist under the environment and some kinds of living beings will die. The environment should be amended as soon as possible. The excess usage of agrochemicals must be stopped. Kitchen garbage from each household should be treated with EM to make them harmless organic fertilizer. It can reduce the total amount of waste which should be burnt. The production of dioxin can be stopped by using EM Technology.

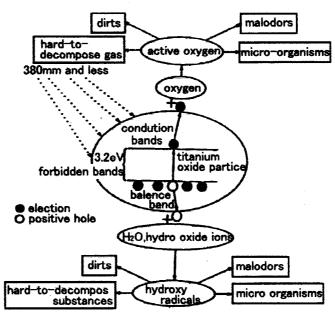


Figure 4. Oxidative Decomposition by the Photocatalyst

# Restoration of Ecosystem and Preservation of the Environment by EM Technology

EM is a mixture of useful microorganisms, which are collected from nature. Therefore, EM Technology is safe and nature-friendly. The various kinds of living organisms like algae, waterweeds, dragonfly, locusts, spiders, leeches, killifish, mud snails, frogs and egrets inhabit in the fields which has been applied with EM even in affected ureas by dioxin. The microbes such as ray fungi and Pseudomonas sp. and algae such as chlorella plus water fleas were also investigated in pot test. It can be shown that EM can change harmful substances, including agrochemicals to harmless forms.

EM can increase the number and variety of microbes. According to the result of the pot

test, various living things could grow for over one year in the 500ml volume of pot, which was added to soil, applied EM and 300ml of water. EM can recover the self-cleaning action of nature.

EM Technology has enough potential for being basic technique of soil amendment and the protection of the environment.

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