Microbial Approach of Rhizoremediation for Soils Contaminated by PAHs

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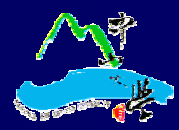
INTRODUCTION

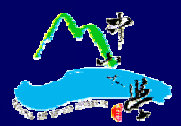
INTRODUCTION

- Polycyclic aromatic hydrocarbons (PAHs) have excellent heat resistant and chemical stability.
- PAHs have extremely high fat-soluble and low biological metabolism properties.
- PAHs are difficult to be biodegraded in the environment.



INTRODUCTION

- PAHs are hazardous materials, which might cause carcinogenesis, mutagenesis, malformation for human beings.
- Therefore, it is important to develop novel and suitable remediation technology to treating PAHs.
- Phytoremediation is an environmental friendly bioremediation technology, which utilizes the absorption, degradation, stabilization and rhizosphere effects of the plant to remediate polluted soils.

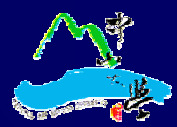


INTRODUCTION

- The advantages of phytoremediation:
  - It is lower cost and lower energy required.
  - It is far less disruptive to the environment.
  - There is no need for disposal sites.
  - It has high probability of public acceptance.
  - It avoid excavation and heavy traffic.
  - It has potential versatility to treat a diverse range of hazardous materials.
  - It may be used in much large scale clean-up.
  - It is environmental friendly ecotechnology.

INTRODUCTION

- The disadvantages of phytoremediation:
  - It needs longer time for remediation due to slow growth of plants.
  - It is limited by climate change and soil characteristics.
  - The plants, especially used for adsorbing heavy metals, still need for disposal.
  - The pollutants may enter onto ground again by litter effects.
  - The plant root exudate may increase the solubility of pollutants to increase their distribution rates in soil environment.

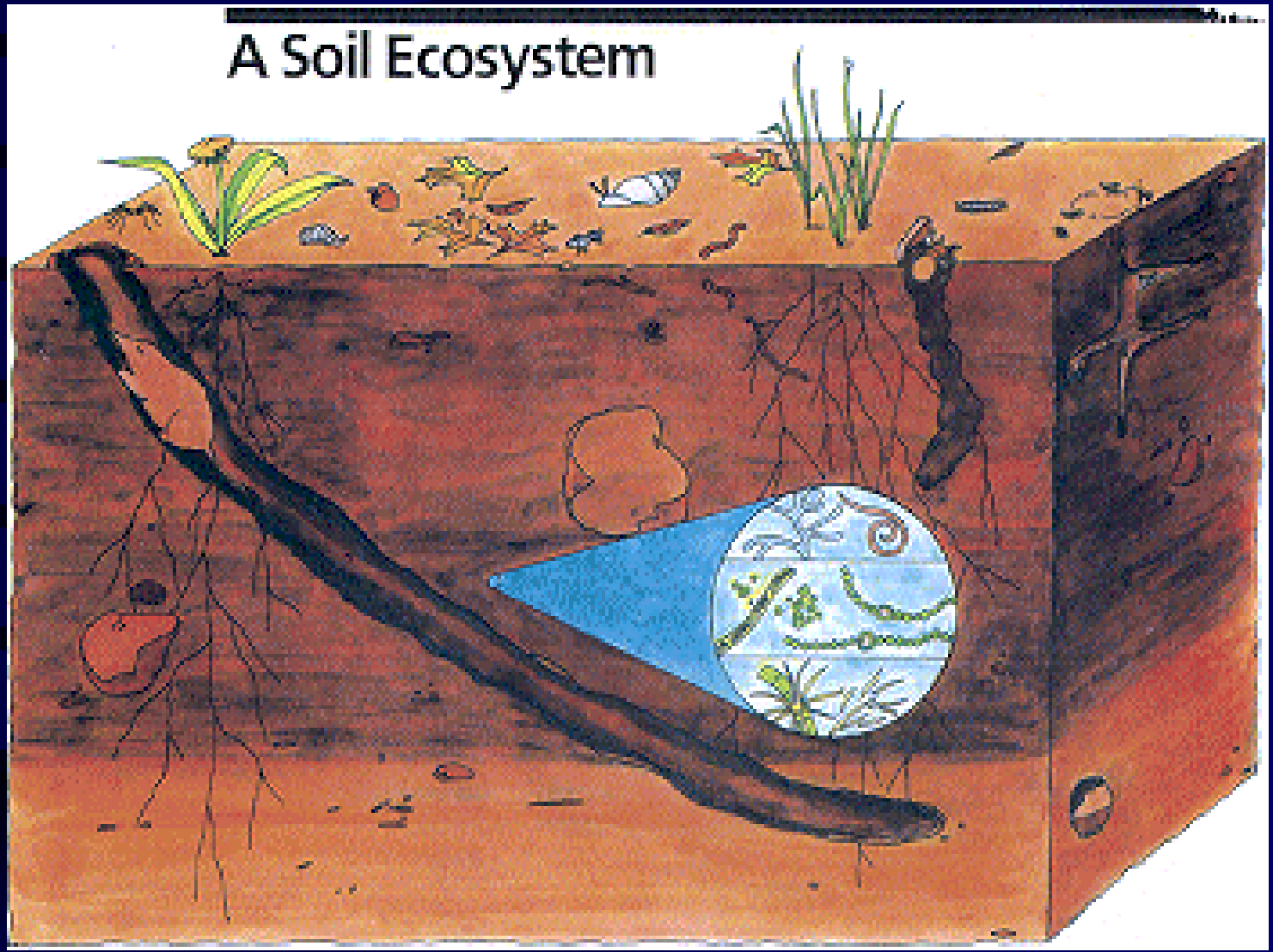


INTRODUCTION

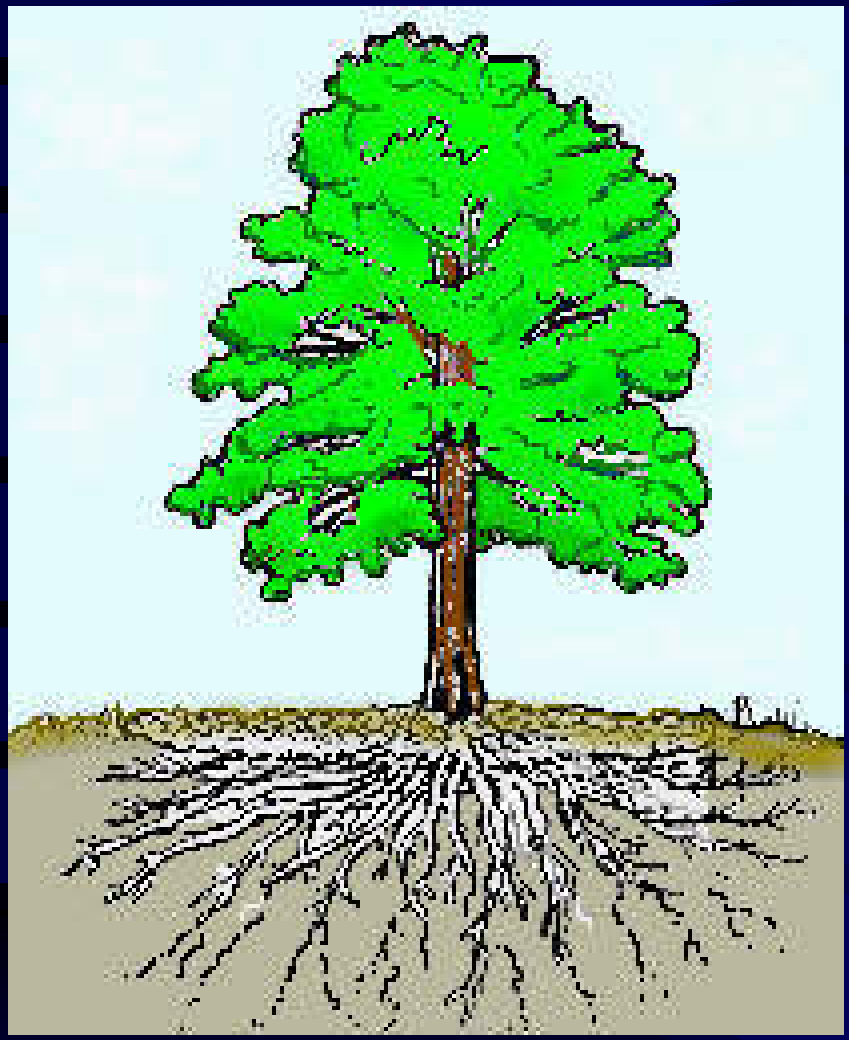
- What is rhizoremediation?

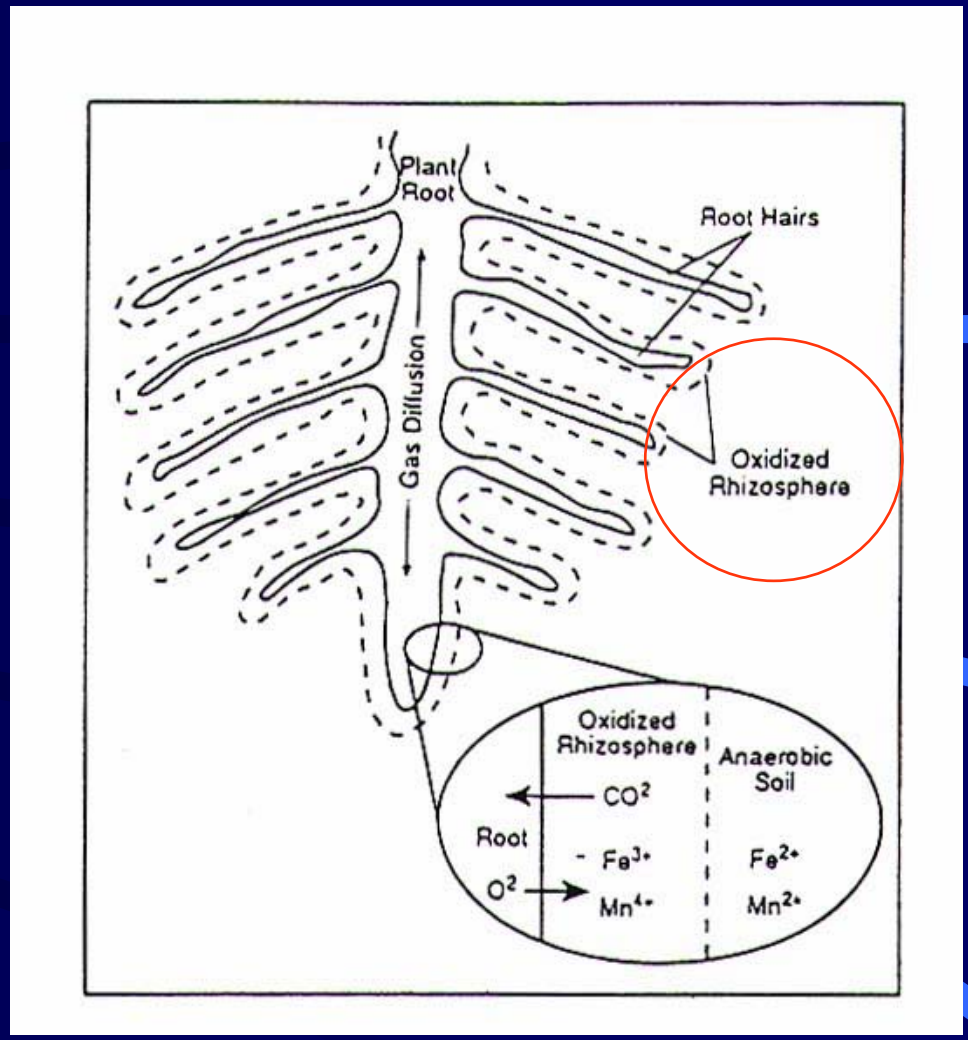
A biological treatment of (organic) contaminants in soils by enhanced bacterial and fungal activity in the rhizosphere of certain vascular plants (Susarla, *et al*., 2002).

Plants and microorganisms often have symbiotic relationships making the root zone or rhizosphere an area of very active microbial activity (Siciliano & Germida, 1998). *eg.* root exudate, enzymes, oxygen



Microbes are very active in rhizopshere of plants





"Root zone effect" is helpful for rhizodegradation of organic contaminates by microbes in rhizosphere

INTRODUCTION

- The purpose of this study?

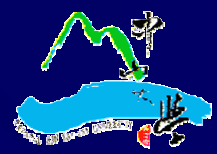
Studying the removal efficiencies of PAHs in soils by rhizoremediation, and further trying to investigate the possible removal mechanismes learned by microbial activities in rhizosphere.

MATERIALS & METHODS

MATERIALS AND METHODS

- Experimental materials:
  - Pyrene: purity 90% (Fluka)
  - Plant species selected:

1. *Phragmites communis* (reeds)
2. *Typha orientalis* (cattails)
3. *Vetiveria zizanioides*
4. *Rohdea japonica*
5. *Cyperus malaccensis* (Salt marsh plant)
6. *Bolboschoenus planiculmis* (Salt marsh plant)
7. *Bidens pilosa*



MATERIALS AND METHODS

Typha orientalis

Rohdea japonica

Vetiveria zizanioides

MATERIALS AND METHODS

Phragmites communis

Bidens pilosa

MATERIALS AND METHODS

Bolboschoenus planiculmis

(Salt Marsh Plant)

Cyperus malaccensis

(Salt Marsh Plant)

MATERIALS AND METHODS

- Experimental materials:
  - Microbial species added into soils for bioaugmentation tests: (10^8 CFU/mL, 10 mL)

1. *Rhizopus sp.* (a)
2. *Rhizopus sp.* (b)
3. *Penicillium sp.*

  - Culturing media used to identify microorganism species in soil:

    SAB, TGA , DHL and TSA

MATERIALS AND METHODS

- Experimental methods:
  - Preparing soil samples contaminated by pyrene:

    Dissolving 0.92 g pyrene in 200 mL acetone, and then poring into each soil sample (3 Kg). Mixing and drying.

MATERIALS AND METHODS

- Experimental methods:

  Experimental procedures:

  – Preparing 30 pots of soil samples contaminated by pyrene.(3 Kg for each)

  – Planting the 7 species of plants mentioned previously in the pots. Each species was prepared for 3 pots, and 3 pots were used as controls.

  – The other 6 pots were sterilized, and then 3 of them were planted with cattails, while 3 of them were used as control tests.

MATERIALS AND METHODS

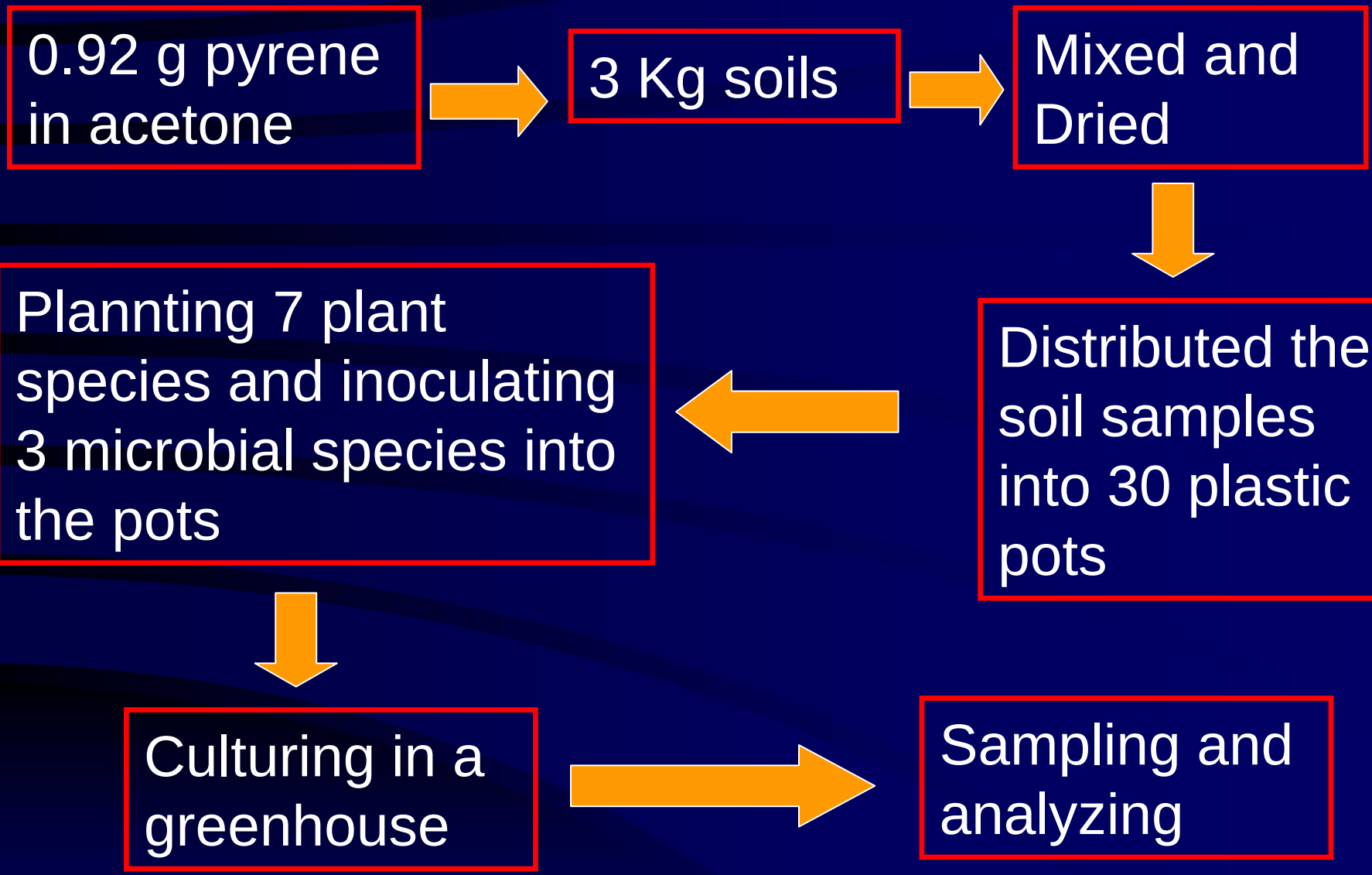
- Experimental methods:

  Experimental procedures:

  – Inoculating three microbial species into the pots with and without vegetation.
  – Put all the pots in a greenhouse for culturing.
  – The soil samples were taken from each pot every two weeks to analyze pyrene and total bacterial number.
  – Molecular biotech analysis (PCR, DGGE)

MATERIALS AND METHODS

The experiments were run in a greenhouse


0.92 g pyrene in acetone
3 Kg soils
Mixed and Dried
Distributed the soil samples into 30 plastic pots
Plannting 7 plant species and inoculating 3 microbial species into the pots
Culturing in a greenhouse
Sampling and analyzing

MATERIALS AND METHODS

- Analytical methods:
  - Extracting soil samples for HPLC:
  1. 2 g soil and 1 g Na_2SO_4 (dehydride soil)
  2. Adding 20 mL CH_2Cl_2 (sonicator 3 min)
  3. Filtration, concentration, and HPLC analysis
  - Conditions set up for analyzing pyrene by using a HPLC:
  1. Columme: C-18
  2. Carry Liquid: Acetonitril
  3. Flow rate: 0.5 mL / min
  4. Injection volume: 10 μL

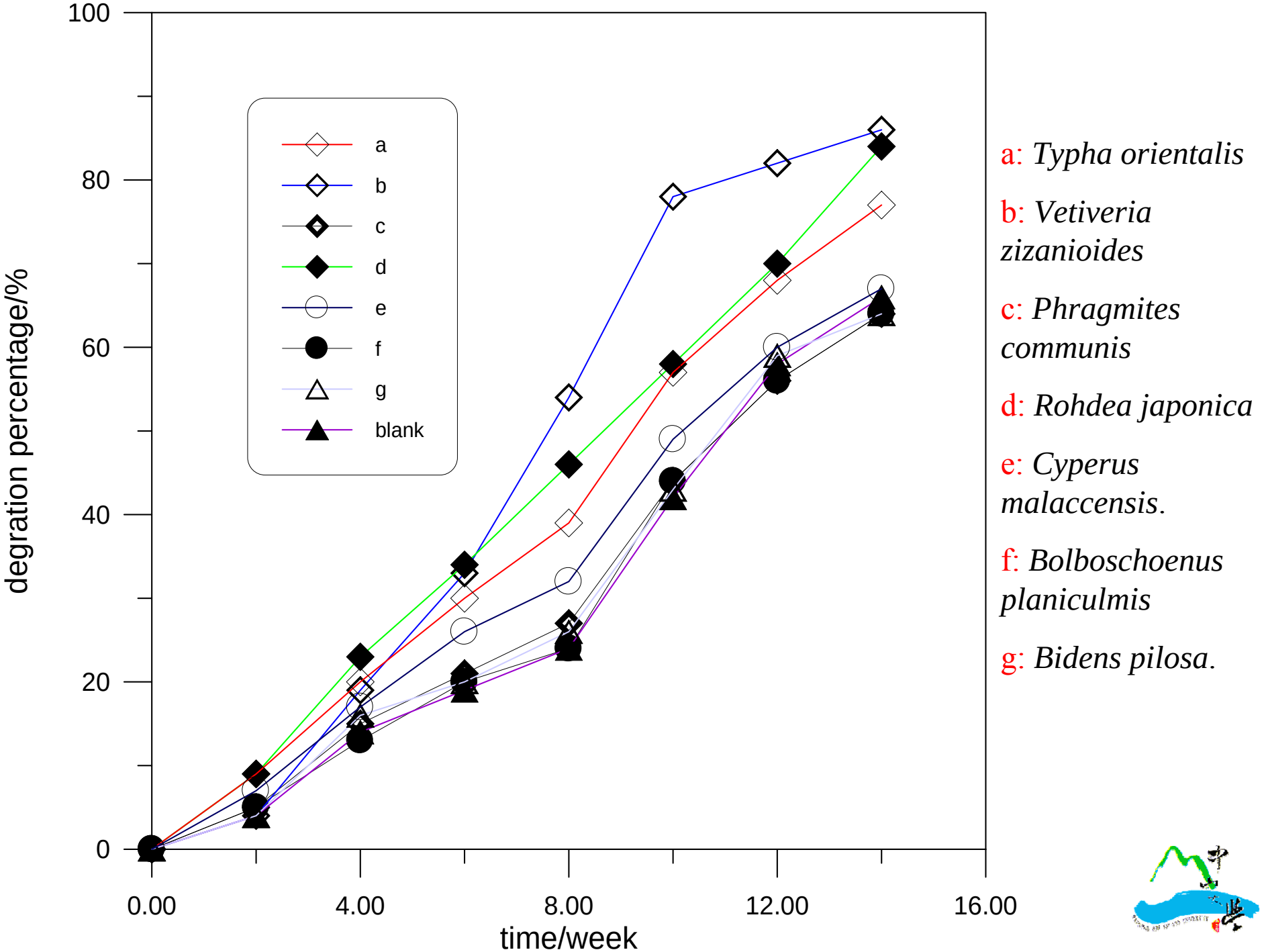
RESULTS & DISCUSSION

RESULTS AND DISCUSSION

The effect of plant species on the pyrene degradation percentage

Pyrene removal ( )	*Typha orientalis*	*Vetiveria zizanioides*	*Phragmites communis*	*Rohdea japonica*	*Cyperus malaccensis*	*Bolboschoenus planiculmis*	*Bidens pilosa*	blank
2 week	9	4	5	9	7	5	4	4
4 week	20	19	15	23	17	13	16	14
6 week	30	33	21	34	26	20	20	19
8 week	39	54	27	46	32	24	26	24
10 week	57	78	44	58	49	44	43	42
12 week	68	82	56	70	60	56	59	58
14 week	77	86	64	84	67	64	64	66

(The original concentration of pyrene in the contaminant soil is 275 mg/Kg)



a: *Typha orientalis*

b: *Vetiveria zizanioides*

c: *Phragmites communis*

d: *Rohdea japonica*

e: *Cyperus malaccensis*.

f: *Bolboschoenus planiculmis*

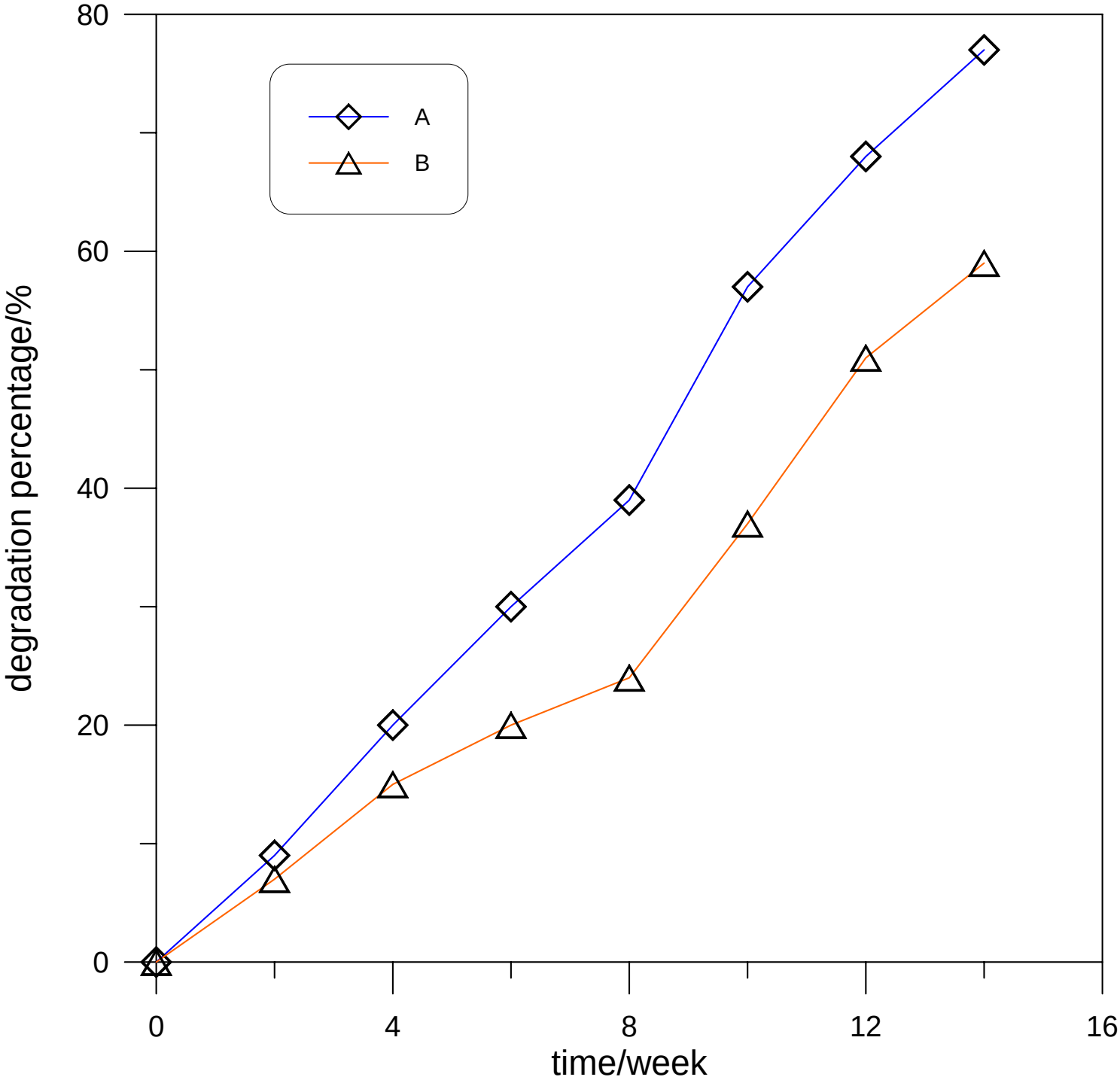
g: *Bidens pilosa*.

RESULTS AND DISCUSSION

The effect of soil sterilization on the pyrene degradation percentage

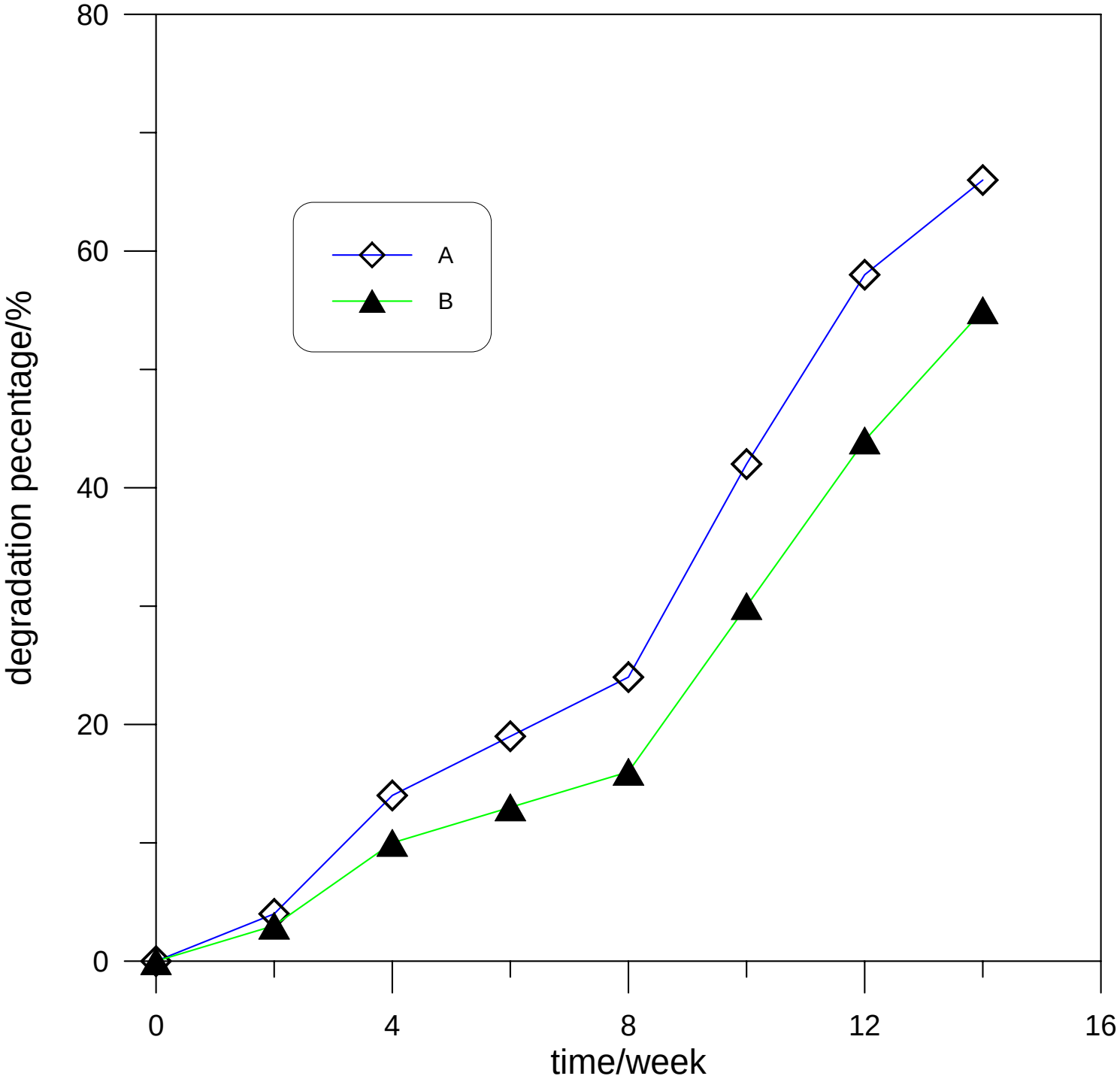
Pyrene removal (%)	*Typha orientalis*	Blank	*Typha oriental* (sterilization)	Blank (sterilization)
2 week	9	4	7	3
4 week	20	14	15	10
6 week	30	19	20	13
8 week	39	24	24	16
10 week	57	42	37	30
12 week	68	58	51	44
14 week	77	66	59	55

(The original concentration of pyrene in the contaminant soil is 275 mg/Kg)



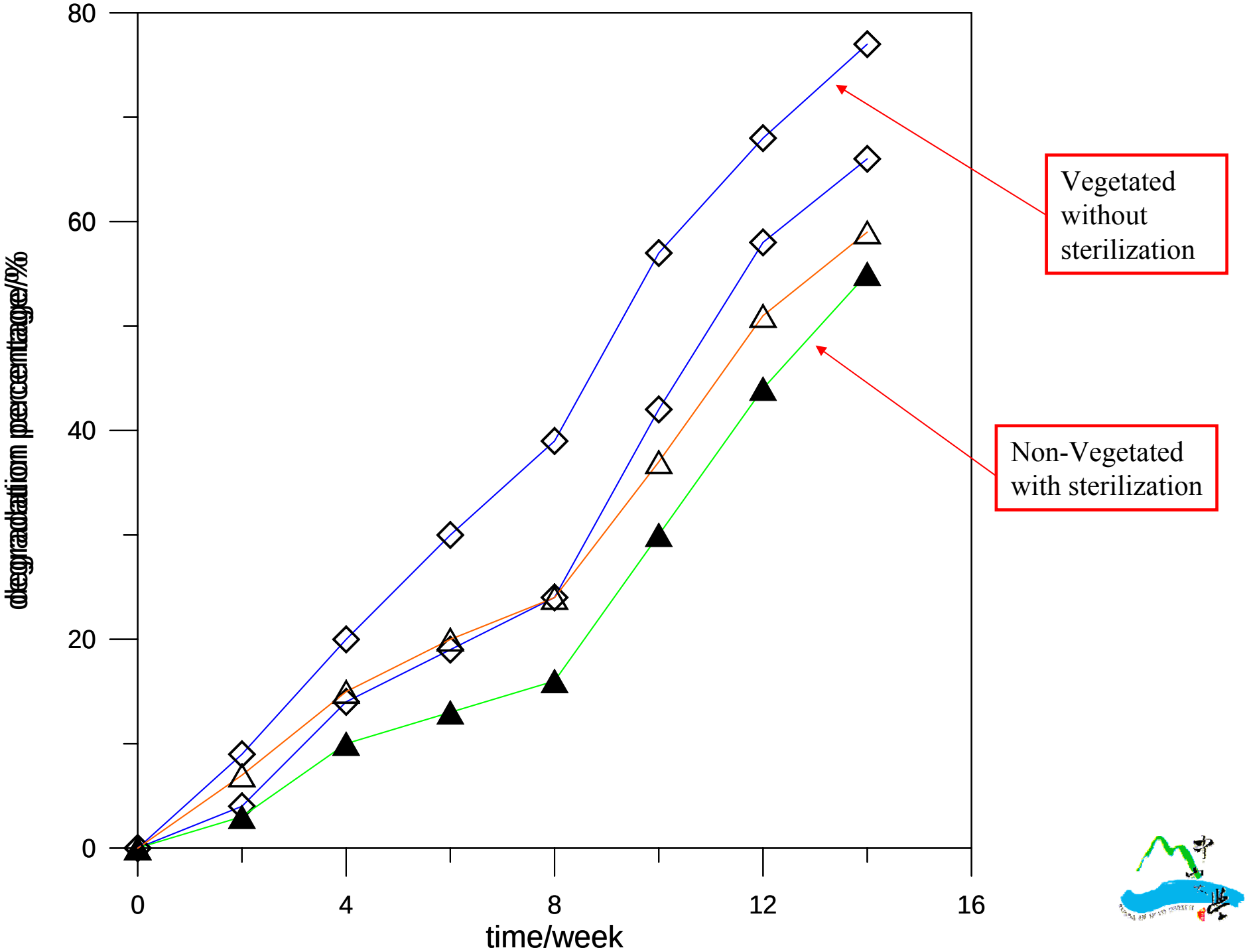
A: Typha without sterilization

B: Typha with sterilization.



A: Non-vegetation without sterilization

B: Non-vegetation with sterilization.


80
60
40
20
0
degradation percentage/%
0
4
8
12
16
time/week
Vegetated without sterilization
Non-Vegetated with sterilization

RESULTS AND DISCUSSION

The influence of total bacterial number by plant species

Total Bacterial Number (CFU/g)	*Typha orientalis*	*Vetiveria zizanioides*	*Phragmites communis*	*Rohd-Ea japonica*	*Cyperus malaccensis*	*Bolboschoenus planiculmis*	*Bidens pilosa*	blank
0 week	$2.0*10^4$	$2.0*10^4$	$2.0*10^4$	$2.0*10^4$	$2.0*10^4$	$2.0*10^4$	$2.0*10^4$	$2.0*10^4$
2 week	$1.2+10^6$	$1.2*10^6$	$5.5*10^5$	$4.0*10^6$	$1.2*10^6$	$6.0*10^5$	$7.3*10^5$	$4.0*10^5$
4 week	$2.0*10^6$	$1.3*10^8$	$1.8*10^5$	$1.6*10^8$	$3.0*10^5$	$1.4*10^5$	$1.1*10^6$	$1.2*10^6$
6 week	$8.1*10^5$	$4.5*10^6$	$3.1*10^5$	$1.7*10^6$	$2.5*10^5$	$1.7*10^5$	$4.1*10^5$	$5.3*10^5$
8 week	$1.3*10^5$	$1.8*10^6$	$4.0*10^5$	$3.0*10^5$	$1.3*10^5$	$3.0*10^5$	$1.2*10^5$	$1.3*10^5$
10 week	$4.0*10^5$	$7.8*10^5$	$4.2*10^5$	$2.6*10^5$	$1.9*10^5$	$2.3*10^5$	$2.7*10^5$	$1.3*10^5$
12 week	$3.6*10^5$	$6.3*10^5$	$3.6*10^5$	$2.5*10^5$	$1.8*10^5$	$1.3*10^5$	$1.8*10^5$	$1.1*10^5$
14 week	$1.8*10^5$	$2.1*10^5$	$2.2*10^5$	$1.1*10^5$	$1.0*10^5$	$1.2*10^5$	$1.3*10^5$	$1.0*10^5$

(The original concentration of pyrene in the contaminant soil is 275 mg/Kg)

RESULTS AND DISCUSSION

The influence of total bacterial number by Soil sterilization

Total Bacterial Number (CFU/g)	*Typha orientalis*	Blank	*Typha Oriental* (sterilization)	Blank (sterilization)
0 week	$4.0*10^4$	$2.0*10^4$	1	1
2 week	$1.2*10^6$	$4.0*10^5$	$6.6*10^4$	$8.7*10^4$
4 week	$2.0*10^6$	$1.2*10^6$	$2.2*10^5$	$2.0*10^5$
6 week	$8.1*10^5$	$5.3*10^5$	$2.1*10^5$	$2.7*10^5$
8 week	$1.3*10^5$	$1.3*10^5$	$1.1*10^5$	$2.9*10^5$
10 week	$4.0*10^5$	$1.3*10^5$	$2.0*10^5$	$2.8*10^5$
12 week	$3.6*10^5$	$1.1*10^5$	$1.5*10^5$	$2.1*10^5$
14 week	$1.8*10^5$	$1.0*10^5$	$1.3*10^5$	$1.5*10^5$

(The original concentration of pyrene in the contaminant soil is 275 mg/Kg)

RESULTS AND DISCUSSION

The effect of selective plant species and additive microorganisms on the pyrene degradation percentage

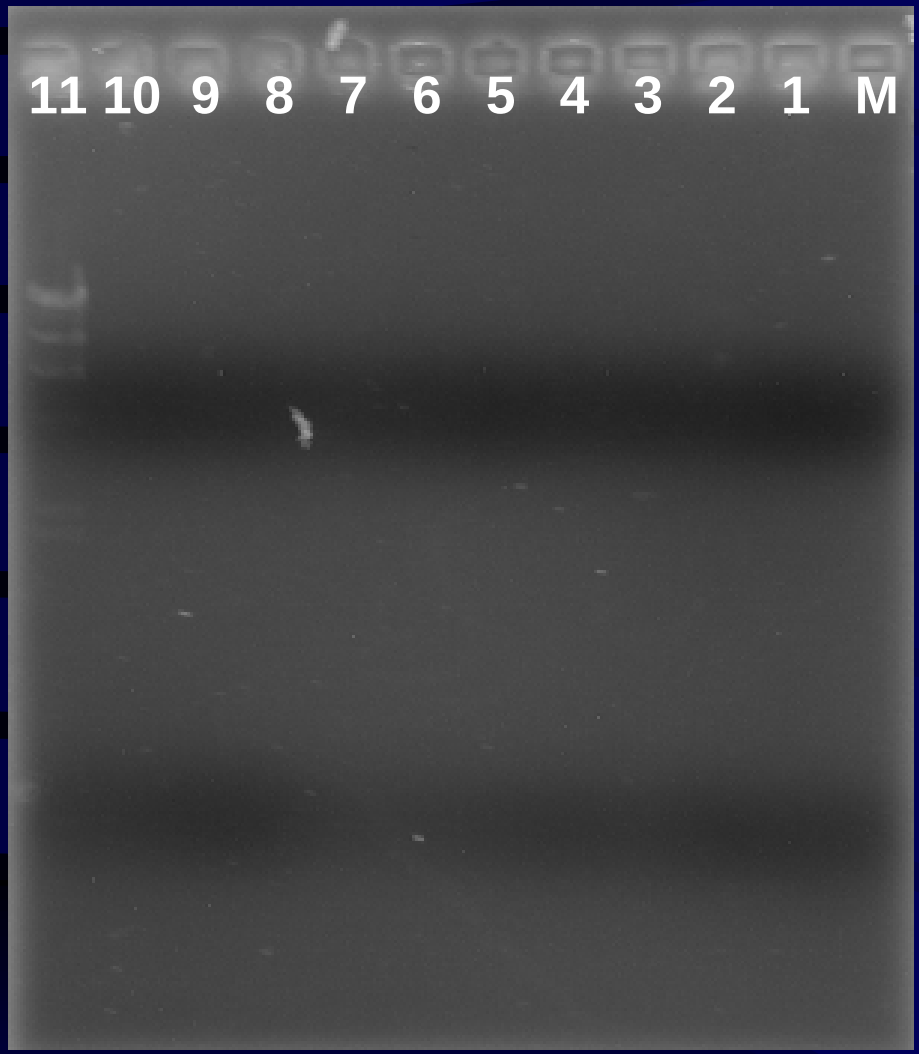
%	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
0 week	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2 week	4	3	4	2	4	4	5	4	5	6	5	3	3	2	3	2
4 week	11	10	13	10	13	12	14	11	14	13	16	12	9	8	10	8
6 week	18	17	19	16	21	21	13	10	25	23	27	24	18	20	18	18
8 week	26	26	28	25	30	28	33	29	36	35	38	34	27	28	26	25
10 week	36	34	36	34	37	34	38	35	43	42	44	40	34	34	31	31

A: with *Typha orientalis*
B: with *Typha orientalis* and *Rhizopus sp.* (a)
C: with *Typha orientalis* and *Rhizopus sp.* (b)
D: with *Typha orientalis* and *Penicillium sp.*
E: with *Vetiveria zizanioides*
F: with *Vetiveria zizanioides* and *Rhizopus sp.* (a)
G: with *Vetiveria zizanioides* and *Rhizopus sp.* (b)
H: with *Vetiveria zizanioides* and *Penicillium sp.*
I: with *Rohdea japonica*
J: with *Rohdea japonica* and *Rhizopus sp.* (a)
K: with *Rohdea japonica* and *Rhizopus sp.* (b)
L: with *Rohdea japonica* and *Penicillium sp.*
M: with *Rhizopus sp.* (a) strain
N: with *Rhizopus sp.* (b) strain
O: with *Penicillium sp.* strain
P: Blank

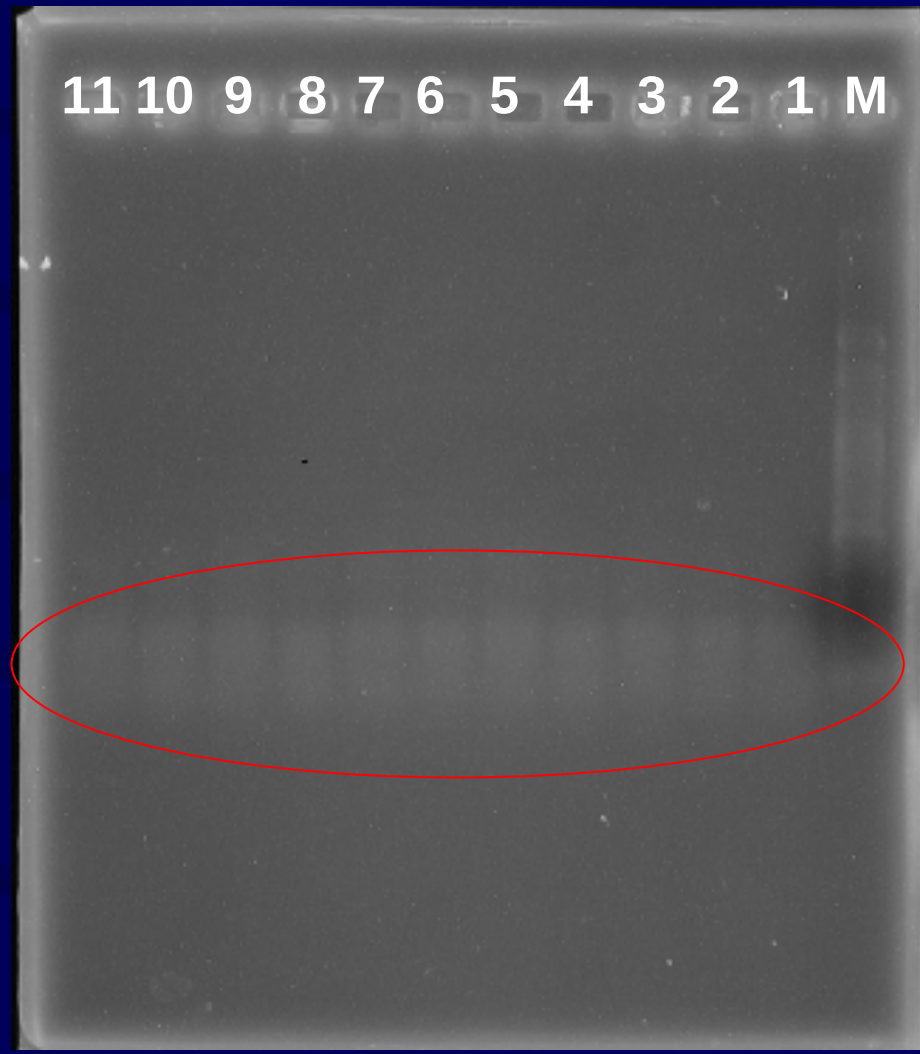
The effect of plant species on the microorganism pyrene

	0 month	1 month	2 mooth
A	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial* *7.Rhibopus sp.*	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial*	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial*
E	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial* *7.Rhibopus sp.*	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *6.Saccharomyces cerevisial* *7.Rhibopus sp.*	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *6.Saccharomyces cerevisial* *7.Rhibopus sp.*
I	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial* *7.Rhibopus sp.*	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial*	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial*
P	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial* *7.Rhibopus sp.*	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial* *7.Rhibopus sp.*	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial* *7.Rhibopus sp.*

	0 month	1 month	2 month
M	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial* *7.Rhibopus sp.* *8.Rhizopus sp. a strain*	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial* *7.Rhibopus sp.*	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial* *7.Rhibopus sp.*
N	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial* *7.Rhibopus sp.* *8.Rhizopus sp. b strain*	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial* *7.Rhibopus sp.*	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial* *7.Rhibopus sp.*
O	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial* *7.Rhibopus sp.* *8. Penicillium sp. strain*	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial* *7.Rhibopus sp.*	*1.Escherichia coli.* *2.Bacillus subtlis* *3.Staphyloccus aureus* *4.Streptococcus sp.* *5.Lactobacillus sp.* *6.Saccharomyces cerevisial* *7.Rhibopus sp.*

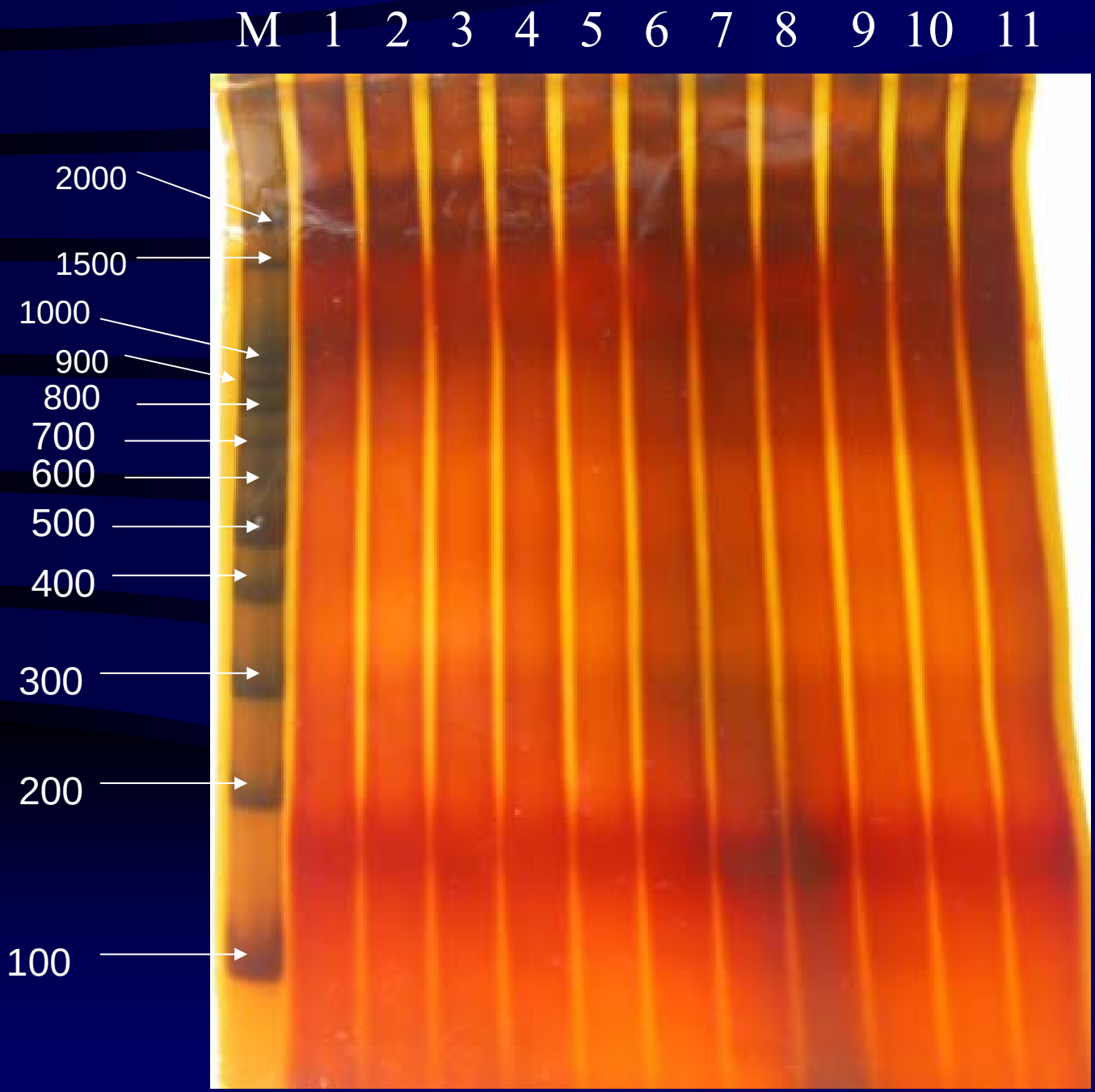


Electrophoresis of chromosome DNA extracted from soil



Electrophoresis of PCR amplified DNA extracted from soil

Lane M λ Hind DNA Marker
Lane 1 initial soil (0 month)
Lane 2 Soil with *Typha orientalis* (after 1 month)
Lane 3 Soil with *Vetiveria zizanioides* (after 1 month)
Lane 4 Soil with *Rohdea japonica* (after 1 month)
Lane 5 Soil without plant (after 1 month)
Lane 6 Soil without plant & without fertilizer (after 1 month)
Lane 7 Soil with *Typha orientalis* (after 2 month)
Lane 8 Soil with *Vetiveria zizanioides* (after 2 month)
Lane 9 Soil with *Rohdea japonica* (after 2 month)
Lane 10 Soil without plant (after 2 month)
Lane 11 Soil without plant & without fertilizer (after 2 month)



Analysis of DGGE by PCR amplified DNA from soil

Lane M 100 bp lander Marker
Lane 1 initial soil (0 month)
Lane 2 Soil with *Typha orientalis* (after 1 month)
Lane 3 Soil with *Vetiveria zizanioides* (after 1 month)
Lane 4 Soil with *Rohdea japonica* (after 1 month)
Lane 5 Soil without plant (after 1 month)
Lane 6 Soil without plant & without fertilizer (after 1 month)
Lane 7 Soil with *Typha orientalis* (after 2 month)
Lane 8 Soil with *Vetiveria zizanioides* (after 2 month)
Lane 9 Soil with *Rohdea japonica* (after 2 month)
Lane 10 Soil without plant (after 2 month)
Lane 11 Soil without plant & without fertilizer (after 2 month)

CONCLUSIONS

CONCLUSIONS

- The results indicated that the removal percentages of pyrene in the pots planted with *Rohdea japonica*, *Typha orientalis* and *Vetiveria zizanioides* were increased more significantly than the others.
- When the pyrene contaminanted soil was sterilized, the removal percentages of pyrene were decreased significantly, no matter with or without plant species.

CONCLUSIONS (cont.)

- The rhizospheric microorganisms surrounded the root were an important factor to the affect Pyrene degradation.
- The plant species in our study, such as *Rohdea japonica*, *Typha orientalis*, and *Vetiveria zizanioides* are suitable plant species to treat PAHs, such as pyrene, contaminanted soil in phytoremediation.

CONCLUSIONS (cont.)

- Molecular biotechnology, such as PCR and DGGE, is helpful to understand the species of rhizopheric microorganisms involved in rhizoremediation of soils contaminated by PAHs, or other organic pollutants.
- Further study is still required for sequence and isolation of those microorganisms in the rhizosphere of plant and analysis of the root exudates in order to understand the relationship between the plants and rhizospheric microorganisms for degradation of PAHs.

THANK YOU

Q & A