Searching for Nature Stories 2018

FunGsorb Capsule

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1. ABSTRACT

In this project, our objectives are to investigate 1) the effect of different concentration of Cd in soil in different field sites in Hong Kong on the infection rate of mycorrhizae in couch grass, 2) soil trapping capability of the root of couch grass in cadmium-contaminated soil model. 3) the effect of arbuscular mycorrhiza fungi on growth of couch grass in cadmium-contaminated soil model. 4) the effect of arbuscular mycorrhiza fungi on concentration of cadmium and essential elements (N, P and K) in soil trapped by the root of couch grass.

We successfully invented a one-step technology, FunGsorb capsule, which could be applied for *on-situ* phytoremediation. Once this invented capsule (with 1g couch grass and 5g arbuscular mycorrhizae fungi seeds) dissolved in soil, the seeds released and germinated. In this experimental setting, 35.24 % of cadmium were removed from the polluted soil in 45 days by the capsule which costs only around $2.

2. Introduction

2.1 Introduction to the research topic

Areas storing abandoned electrical appliances or surface runoff from e-waste dumpling from Pearl River Delta contain contaminated soil with high concentration of heavy metals. Heavy metal contamination in soil raised concerns over their potential effects on human health and the environment. Chronic inhalation or oral exposure to heavy metal such as Cadmium (Cd) leads to kidney failure. In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by the heavy metal as they can enter the food chain via agricultural products or contaminated drinking water by the mechanism of bioaccumulation. (Bradl, 2002)

Couch grass is abundant and native to Hong Kong. If it can be infected by mycorrhizae and used in phytoremediation, it would be used to solve the ecological and health problem mentioned above.

2.2 Objectives

1. To investigate the effect of different concentration of Cd in soil in different field sites in Hong Kong on the infection rate of mycorrhizae in couch grass
2. To investigate the effect of arbuscular mycorrhiza fungi on soil trapping capability of the root of couch grass in cadmium-contaminated soil model.
3. To study the effect of arbuscular mycorrhiza fungi on growth of couch grass in cadmium-contaminated soil model.
4. To study the effect of arbuscular mycorrhiza fungi on concentration of cadmium and essential elements (N, P and K) in soil trapped by the root of couch grass.

2.3 Research questions

1. How does different concentration of Cd in soil affect the infection rate of mycorrhizae in couch grass in different field sites in HK?
2. How does mycorrhizae affect the soil trapping capability of the root of couch grass in Cd contaminated soil?
3. How does mycorrhizae affect the growth of couch grass in Cd contaminated soil in terms of mass?
    - Does mycorrhizae help trapping essential minerals (N, P, K) in soil which in turn affects the growth of the grass?
    - Does mycorrhizae help synthesize chlorophyll which in turn affects the growth of the grass?
4. How does mycorrhizae affect the concentration of Cd, essential elements (N, P, K) in soil trapped by the root of couch grass?
    - What is the efficiency of removing Cd in soil?
    - What is the percentage of essential elements (N, P, K) trapped by the root of different groups?

2.4 Backgroud information of *Cynodon dactylon*

2.4.1 Classification (Bradl H, 2002)

Kingdom	Plantae
Phylum	Spermatophyta
Class	Monocotyledonae
Order	Cyperales
Family	Poaceae
Genus	Cynodon
Species	Cynodon dactylon

Figure 1.1 Photo of couch grass

2.4.2 Description

Couch grass is widely naturalized in tropical and subtropical regions of the world. It is a native herbaceous plant in Hong Kong. It is a fast growing grass that spreads by seeds and stolons and rapidly colonizes new areas and grows forming dense mats. It is very drought tolerant by virtue of rhizome survival through drought-induced dormancy over periods of up to 7 months. It has the ability to easily re-sprout from stolons and rooted runners. (Hrishikesh et al, 2010) Plants also recover quickly after fire and can tolerate at least several weeks of deep flooding. (Cook et al., 2005)

2.5 Backgroud information of *Glomus mosseae*

2.5.1 Classification (Bradl H, 2002)

Kingdom	Fungi
Phylum	Glomeromycota
Class	Glomeromycetes
Order	Glomerales
Family	Glomeraceae
Genus	Glomus
Species	*Glomus mosseae*

Figure 1.2 Photo of arbuscular mycorrhiza fungi (500X)

2.5.2 Description

An arbuscular mycorrhiza fungus is a fungus which can have symbiotic relationship with specific plants. They are reported to be present on the roots of plants growing on metal-contaminated soils. During symbiotic interaction the hyphal network functionally extends the root system of their hosts and bind heavy metals to polyphosphates within the hyphae. (Leung HM et al, 2006)

2.6 Principal of the experiment

To investigate the effect of different concentration of Cd in soil in different field sites in Hong Kong on the infection rate of mycorrhizae in couch grass. We collected samples from three places: Jordon Valley, Kowloon Park and Yung Yuen Road, Yuen Long. It was suspected that Yung Yuen Road should have soil contaminated with Cd as there is a recycling market nearby. In Jordon Valley and Kowloon Park, there is no observable source of heavy metal pollution. We collected soil samples from the three field sites and observed the infection rate of mycorrhiza in couch grass under microscope upon staining of the roots.

In another experiment, we measured the mass of soil trapped by root of couch grass with or without arbuscular mycorrhiza fungi in Cadmium polluted soil and unpolluted soil after 45 days of incubation in greenhouse. If the soil detached from the root of couch grass living with arbuscular mycorrhiza fungi is greater in mass, it means the association can help trap more soil in root. The net mass of plant, without trapped soil, would also be measured after soil is separated by DI water. The greater the mass of the plant, the better the growth of the plant is.

To confirm that arbuscular mycorrhiza fungi can really inhabit on the root of couch grass, the root of the couch grass was stained and observed under a microscope to estimate the infection rate by the fungi. The higher the infection rate, the better the condition for arbuscular mycorrhiza fungi and couch grass to have symbiotic relationship. As our group suspected that the growth of a plant can be reflected in the concentration of chlorophyll as well, the total concentration of chlorophyll (chlorophyll a + b) in leaves in each pot were measured by using UV spectrometer.

To assess if couch grass can concentrate Cadmium around the root when growing with arbuscular mycorrhizae fungi, it is necessary to measure the concentration of Cadmium in the soil separated from the root of the plant from each group. Inductively Coupled Plasma Spectrometry (ICP) would be used in measuring the concentration of Cadmium and percentage of essential minerals like N, P and K in the soil. The technique makes use of the wavelengths of light specifically absorbed by an element. They correspond to the energies needed to promote electrons from one energy level to another, higher, energy level (R. Levinson, 2018) The higher the concentration of Cadmium trapped in the soil, the higher the efficiency of removing Cadmium in soil after harvesting the couch grass.

3. Methodology

3.1 Field Study

	Date	Time	Venue	Event
1st field study	2018-4-7	10:00 – 12:00	Jordan Valley Park	Collection of 4 sets of couch grass and soil sample randomly.
2nd field study	2018-4-14		Kowloon Park	
3rd field study	2018-4-21		Yung Yuen Road, Yuen Long	

We chose Yung Yuen Road, Yuen Long as we suspected that it would have higher heavy metal concentration in soil when compared with the other two field sites. Yung Yuen Road has metal recycling market nearby and it has a lot of e-waste abandoned. E-waste is one main source of heavy metal pollution. Kowloon Park and Jordan Valley Park should have lower chance of having heavy metal pollution as there are no industries and metal recycling market nearby.

Figure 1.3 metal recycling market I Yung Yuen Road, Yuen Long

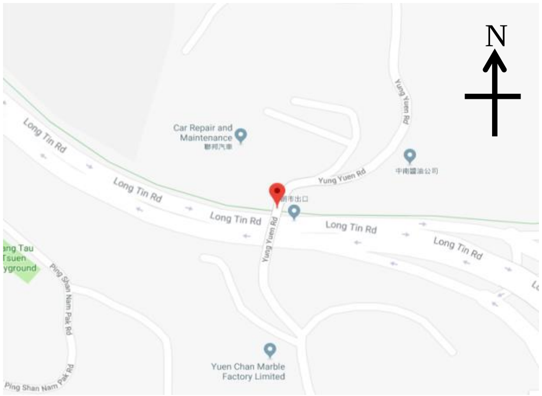

Figure 1.4 Location of Yung Yuen Road, Yuen Long

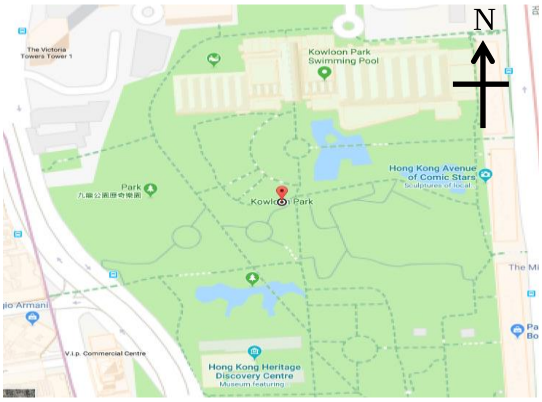

Figure 1.5 Location of Kowloon Park

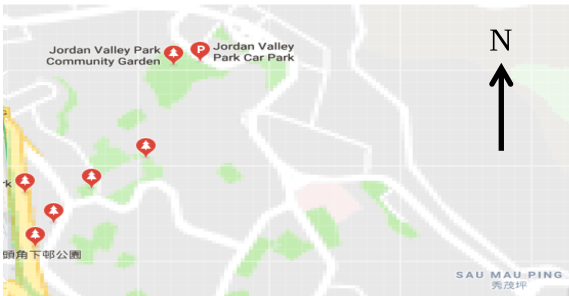

Figure 1.6 Location of Jordan Valley Park

3.2 Objective 1: To investigate the effect of different concentration of Cd in soil in different field sites in Hong Kong on the infection rate of mycorrhizae in couch grass

3.2.1 Procedure:

Staining and microscopic study of arbuscular mycorrhiza fungi

1. 50 roots from each samples collected in each field sites were washed with DI water to remove all soil particles adhered on the roots of couch grass.
2. Fine lateral roots were cut into 2 cm pieces, heated for 20 mins at 100^{o}C in 10% potassium hydroxide to remove cytoplasm. (Figure 2.1)
3. The roots were stained by lactophenol blue.
4. The stained root were then mounted on glass slides (10 pieces of root per slide) for examination under a compound microscope (100x to 400x) mounted with an eyepiece cross-hair which could be moved to randomly selected positions.
5. Colonization percentage of mycorrhizae was estimated for each sample by examining 2-cm long pieces of roots (Requena et al. 1996). The root is approximately divided into 4 even parts during observation, if mycorrhiza is observed in one part, then the infection rate is 25%,. If mycorrhiza is observed in two parts, then the infection rate is 50%..etc

Figure 2.1 Boiling of roots in potassium hydroxide.

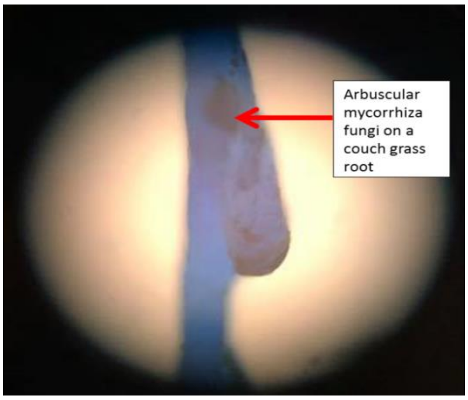

Figure 2.2 Colonization of mycorrhizae on root.

Analysis of the concentration of Cd in soil collected in different field sites

1. Soil separated from the root of couch grass in each pot was digested by concentrated hydrochloric acid and 50% HF: con. $HNO_{3,}$ 1:2 (v/v) (USEPA method 3052A) and 1M DTPA [1.976g DTPA: 14.29g triethanolamine; 1.47 g $CaCl_2$ $2H_2O$ and dissolved in 980 ml deionized H_2O and made up to 1L, pH 7.30] (Lindsay and Norvell, 1978) respectively.
2. The Cd concentrations in the soil were determined by Inductively Coupled Plasma Spectrometry (ICP) (Jones and Case, 1990)

3.3 Objective 2: To investigate the effect of arbuscular mycorrhiza fungi on soil trapping capability of the root of couch grass in cadmium-contaminated soil model;

3.3.1 Procedure:

Soil preparation for incubation

1. "Clean" soil was prepared by passing through a 2mm sieve, sterilized by autoclaving at 120^{o}C for 2 hours followed by air-dying.
2. The soils were allowed to equilibrate for a period of 10 days in the greenhouse.
3. All treated soils were added with mineral nutrients: 300 mg nitrate (2 ml of 0.1M calcium nitrate), 50mg phosphorus (0.3 ml of 0.1 M calcium hydrogen phosphate) and 200 mg potassium (1.3 ml of 0.1 M potassium sulfate) as a basal application (Hewitt, 1966).

Setting up of experimental and control pots

1. Four groups of pots were set up as below:

Set up	Content
Group 1 (pot x 4)	5g of arbuscular mycorrhiza fungi seed + 1g of couch grass seed + 500g of sieved soil + 2mg/kg solution form of Cadmium
Group 2 (pot x 4)	5g of arbuscular mycorrhiza fungi seed + 1g of couch grass seed + 500g of sieved soil
Group 3 (pot x 4)	5g of sterilized arbuscular mycorrhiza fungi seed + 1g of couch grass seed + 500g of sieved soil + 2mg/kg solution form of Cadmium
Group 4 (pot x 4)	5g of sterilized arbuscular mycorrhiza fungi seed + 1g of couch grass seed + 500g of sieved soil

Figure 2.3 Set up of pots in greenhouse

2. Pots were incubated in greenhouse with controlled condition (as shown below) for 45 days. The positions of the pots were randomly swapped every day during incubation.
   - Temperature 18-25^{o}C
   - Relative Humidity: 60-80%
   - Light Intensity: 8000 lux
   - All the pots were added with DI water every 2 days to maintain 70% water holding capacity.

Harvesting of plants for measurement

Figure 2.4 Rinsing grass with DI water.

1. On day 45, all couch grass with trapped soil was harvested from each pot.
2. The grass with trapped soil was blotted dry with tissue paper and weighed.
3. The grass with trapped soil was put in DI water. (Figure 2.4)
4. The soil and plants were separated by forceps softly to prevent plant tissue being torn away.
5. The grass were blotted dry with tissue paper and weighed again.
6. Mass of the trapped soil was calculated by the formula below:

Mass of plant with soil trapped – Mass of plant without soil trapped

3.4 Objective 3: To study the effect of arbuscular mycorrhiza fungi on growth of couch grass in cadmium-contaminated soil model.

3.4.1 Procedure:

Measuring concentration of chlorophyll to find out how mycorrhiza and Cd affect the amount of chlorophyll in couch grass (Arnon, et al, 1949).

1. Chlorophyll in 5g of leave in each set up was extracted with 5ml extracting solution (80% aqueous acetone and 2.5mM sodium phosphate buffer, pH7.8, in 1:1) by grinding it with pestle and mortar
2. The mixture was spun down at 35,000 x g for 10 min (Figure 2.3)
3. The liquid layer was then analyzed by an Ultraviolet-visible spectroscopy by measuring the absorbance at 645nm and 663nm. The concentration of chlorophyll a and b pigments were calculated quantitatively according to the following equation: (Arnon, et al, 1949)

$$\textbf{Chlorophyll a (mg/mL)} = 12.7\,A_{663} - 2.69\,A_{645}$$
$$\textbf{Chlorophyll b (mg/mL)} = 22.9\,A_{645} - 4.68\,A_{663}$$

Where:

A_{645} = absorbance at a wavelength of 645 nm;

A_{663} = absorbance at a wavelength of 663 nm;

4. Calculation of the total concentration of chlorophyll in leave:

Total chlorophyll (mg/mL) = Chlorophyll a + Chlorophyll b

Figure 2.5 Extraction of chlorophyll.

Staining and microscopic study of arbuscular mycorrhiza fungi to ensure symbiotic association really takes places in experimental groups

1. 50 roots from each group were washed with DI water to remove all soil particles adhered on the roots.
2. Fine lateral roots were cut into 2 cm pieces, heated for 20 mins at 100^{o}C in 10% potassium hydroxide to remove cytoplasm. (Figure 2.4)
3. The roots were stained by lactophenol blue.
4. The stained root were then mounted on glass slides (10 pieces of root per slide) for examination under a compound microscope (100x to 400x) mounted with an eyepiece cross-hair which could be moved to randomly selected positions. (Figure 2.4)
5. Colonization percentage of mycorrhizae was estimated for each sample by examining 2-cm long pieces of roots (Requena et al. 1996).

3.5 Objective 4: To study the effect of arbuscular mycorrhiza fungi on concentration of cadmium essential elements (N, P and K) in soil trapped by the root of couch grass.

3.5.1 Procedure

Analysis of the concentration of Cd and other essential minerals in soil trapped by root of the grass

Repeat procedure 3.2.1 by Inductively Coupled Plasma Spectrometry (ICP) to determine the concentration of Cd, N, P and K in soil trapped by the root of couch grass (Jones and Case, 1990).

4. Results

4.1 Results on Objective 1: To investigate the effect of different concentration of Cd in soil in different field sites in Hong Kong on the infection rate of mycorrhizae in couch grass

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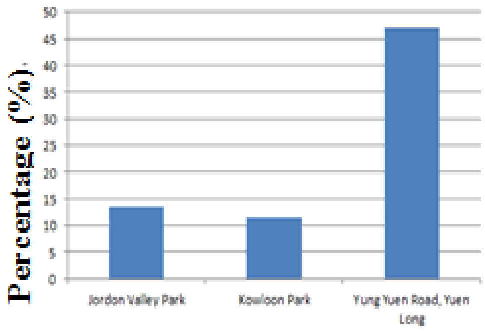

Figure 3.1 Percentage infection of couch grass by arbuscular mycorrhiza fungi in plant in different field sites

This figure shows that the percentage infection of couch grass is the highest in Yung Yuen Road, the one with more Cd pollution in soil. The infection rate of Jordon Valley Park and Kowloon Park are similar.

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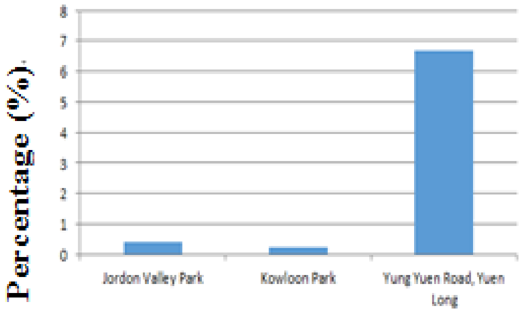

Figure 3.2 Mean concentration of Cadmium (mg/kg) in different field sites. It was found that the concentration of Cd in Yung Yuen Road is obviously higher than that of Jordon Valley Park and Kowloon Park.

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4.2 Results on Objective 2: To investigate the effect of arbuscular mycorrhiza fungi on soil trapping capability of the root of couch grass in cadmium-contaminated soil model

Figure 3.3 Growth of Couch Grass in different groups after 45 days

From left to right row: Group 1: cadmium + couch grass seed + arbuscular mycorrhiza fungi seed x 4. Group 2: couch grass seed + arbuscular mycorrhiza fungi seed x 4. Group 3: cadmium + couch grass seed x 4. Group 4: couch grass seed

This figure obviously shows that the group with cadmium and arbuscular mycorrhiza fungi grows best.

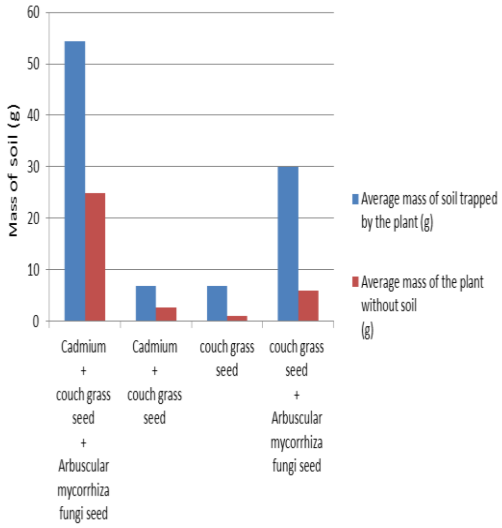

Figure 3.4 Average mass of soil trapped and biomass of the plant in different groups The figure shows that with the presence of cadmium, the amount of soil trapped and the biomass of the plant with the arbuscular mycorrhiza fungi would be higher than those without the fungi. With the absence of cadmium, the amount of soil trapped and the biomass of the plant with the arbuscular mycorrhiza fungi would also be higher than the one without the fungi.

4.3 Results on Objective 3: To study the effect of arbuscular mycorrhiza fungi on growth of couch grass in cadmium-contaminated soil model.

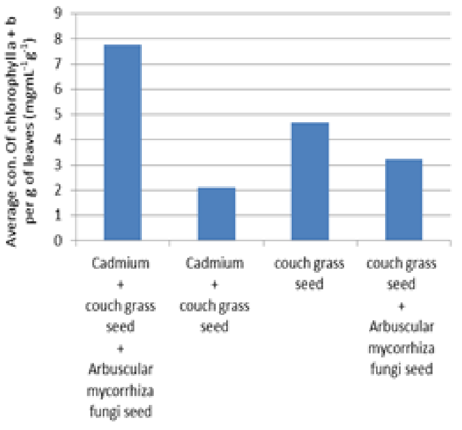

Figure 3.5 Average concentrations of chlorophyll a + b (mgmL^{-1}g^{-1}) in leaves of the plant in different groups.

The figure shows that the average concentration of chlorophyll a and b would be the highest with the presence of both arbuscular mycorrhiza fungi and cadmium.

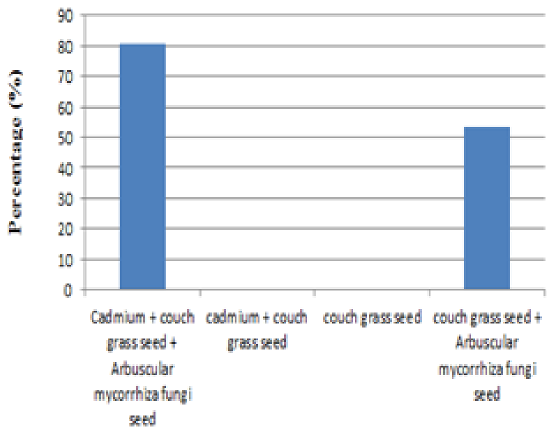

Figure 3.6 Percentage infection of couch grass by arbuscular mycorrhiza fungi in grass in different groups

In the experiment, it was observed that the infection by the fungi would be higher if cadmium is present.

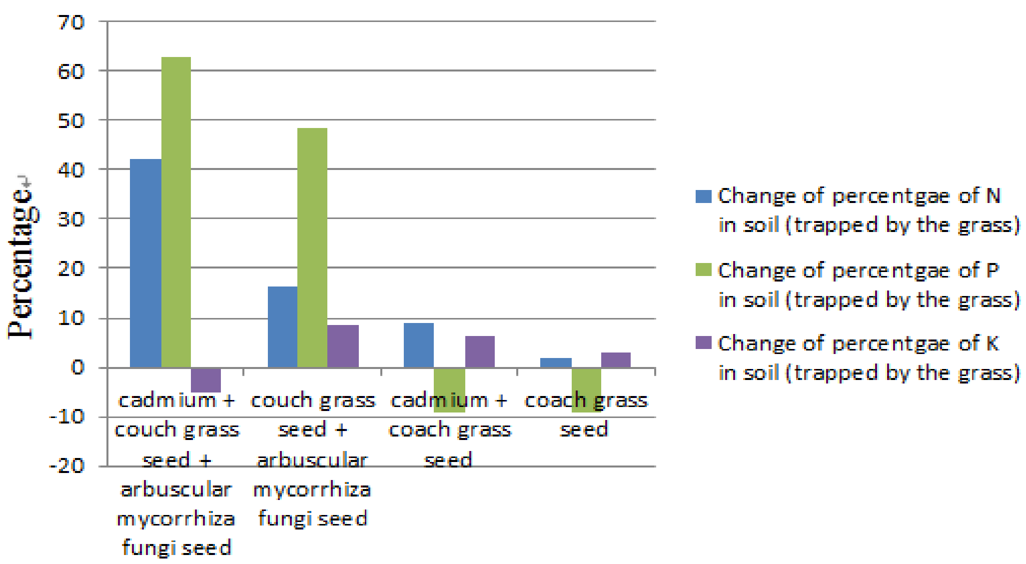

Figure 3.7 Change of percentage of essential minerals (N, P, K) in soil trapped by root of coach grass in different groups. The figure shows that mycorrhiza can help the grass to concentrate N and P in the trapped soil. The percentage is even higher when cadmium is present.

4.4 Results on Objective 4

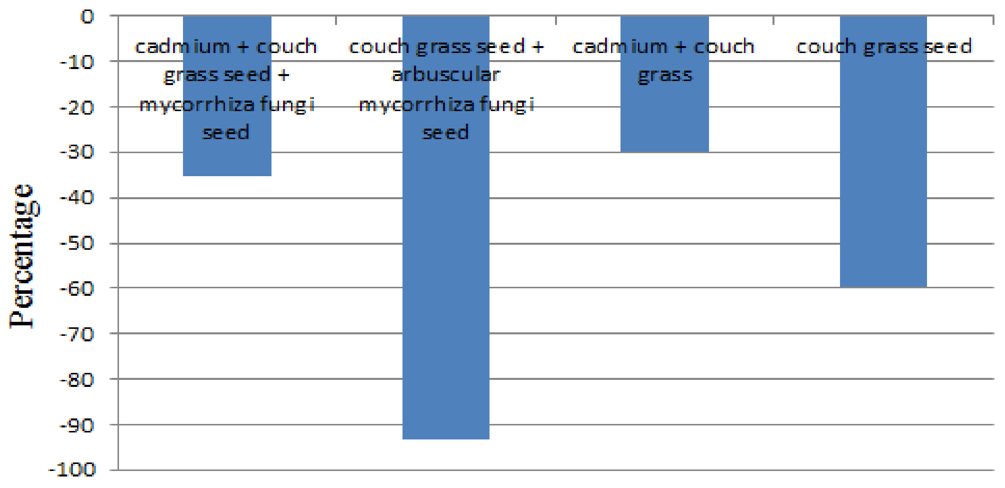

Figure 3.7 Cd contamination change (%) in soil after harvest in different groups. This figure shows that the concentration of cadmium can be removed by 35.24% by the fungi and couch grass in highly polluted soil (with the addition of cadmium before the experiment) and 93.59% in non-polluted soil (without addition of cadmium before the experiment).

5. Discussion

5.1 Objective 1: The effect of different concentration of Cd in soil in different field sites in Hong Kong on the infection rate of mycorrhizae in couch grass

The Cd concentration in soil and infection rate of mycorrhiza in root in couch grass Yung Yuen Road are obviously higher than that of Kowloon Park and Jordon Valley Park. Therefore it shows that the infection rate may increase with the Cd concentration in soil. To confirm that the infection rate is not due to other factor, further find out the effect of mycorrhiza on growth of couch grass and ability of trapping Cd in root, another experiment is needed.

5.2 Objective 2: The effect of arbuscular mycorrhiza fungi on soil trapping capability of the root of couch grass in cadmium-contaminated soil.

In the presence of Cadmium, the average amount of soil trapped by root of couch grass living with arbuscular mycorrhiza fungi (54.3g) is higher than that of the one without the fungi (6.8g). While, in the absence of Cadmium, the average amount of soil trapped by root of couch grass living with arbuscular mycorrhiza fungi (30.0g) is greater than that of the one without the fungi (6.9g). It reflects that the fungi can help the couch grass to trap more soil around the root, the extent is even greater when Cadmium is present. This result supports our hypothesis that during symbiotic interaction the hyphal network functionally extends the root system of their hosts (Hrishikesh, et al, 2010). The higher surface area of the root may allow the host plants to trap more soil. The result also shows that the amount of soil trapped would be much higher if the heavy metal Cadmium and the fungi are present at the same time. This finding reflected that Cadmium and fungi have a combined effect that helps couch grass to grab the greatest amount of soil in root. This result is encouraging as it means more soil polluted with Cadmium can be removed when we harvest the couch grass.

5.3 Objective 3: The effect of arbuscular mycorrhiza fungi on growth of couch grass in cadmium-contaminated soil.

In the presence of Cadmium, the average mass of the plant living with arbuscular mycorrhiza fungi (24.85g) is higher than that of the one without fungi (2.569g). However, in the absence of Cadmium, the average mass the plant living with arbuscular mycorrhiza fungi (5.85g) is higher than that of the one without fungi (1.065g). This result reflects that the fungi can help the couch grass grow faster and better, the extent is even greater when Cadmium is present.

From the data, it was observed that arbuscular mycorrhiza fungi can help couch grass grow faster and better. Again, it may be due to the fact that the root system of the grass is extended by the fungi. The high surface area of the root helps the grass absorb more minerals and nutrients from the soil and thus grows better. It may also be due to the fact that the fungi is small enough to help the root penetrate through space between soil particles and thus better absorption of water and minerals from soil. Again an unexpected finding was observed that grass grows especially well when Cadmium and the fungi are present at the same time. This finding is encouraging as it means when we use couch grass to clean up heavy metal in soil with arbuscular mycorrhizae fungi, the grass can grow faster. The rate of phytoremediation can be higher with faster growth of the grass. It was suspected that Cadmium can help synthesis of chlorophyll of couch grass when arbuscular mycorrhiza fungi is present, and so causes fastest growth of the plant (Leung HM et al, 2006). The experimental results support the saying as it showed that the total concentration of chlorophyll is the highest when both arbuscular mycorrhiza fungi and Cadmium are present. The high concentration of chlorophyll may be the reason of fast growth of couch grass as it may lead to higher rate of photosynthesis. The high infection rate in two groups added with mycorrhiza seed indicates that the infection is successful.

5.4 The effect of arbuscular mycorrhiza fungi on concentration of cadmium and essential elements (N, P and K) in soil trapped by the root of couch grass.

When compared with the concentration of cadmium before and after the experiment, it showed that the couch grass alone can help remove cadmium in soil after harvest, but the efficiency can be greatly increased when arbuscular mycorrhiza fungi is present.

When the concentration of cadmium is low (group II and IV with no addition of cadmium solution), the efficiency of removing cadmium can be increased from 59.79% to 93.59% in 45 days. When the concentration of cadmium is high (group I and III with addition of cadmium solution), the efficiency of removing cadmium can be increased from 29.93% to 35.24% in 45 days.

From this data, it reflected that couch grass and arbuscular mycorrhiza fungi was quite efficient in removing cadmium in polluted soil at a low cost. With lower concentration of Cadmium in soil, the efficiency of removing cadmium from polluted area by growing them together is higher. When applying this technology in removing cadmium in highly polluted soil in the real case, more time is needed to ensure cadmium is removed at a certain level.

It was also found that the percentage of essential elements N and P in soil trapped by couch grass root are higher when the fungi are present. The percentage is the highest when cadmium is present as well (N: 42.1%; P:62.9%). It may explain why the grass growth rate with arbuscular mycorrhiza is higher when compared with those without fungi and the one with cadmium can growth fastest. Phosphorus is a component in nucleic acid which is essential when doing cell division during growth. Nitrogen is an essential element for building chlorophyll, the high percentage of nitrogen found in soil trapped by couch root, when cadmium and arbuscular mycorrhiza fungi is present, may explain the highest concentration of chlorophyll found in the experiment.

5.5 Further Investigation

1. What is the combination of having the highest rate of removal of cadmium in soil (amount of arbuscular mycorrhiza fungi seeds, mass of couch grass seed, mass of soil used and concentration of cadmium in soil)?

   - We have proved that the concentration of cadmium can be removed by 35.24% when 2mg/kg of cadmium was added in this setting. However, it would be worth finding out the combination in which the rate of removal of cadmium would be highest in different concentration of cadmium in soil.
2. How is the performance of removing other heavy metal from arbuscular mycorrhiza fungi and couch grass?

   - There are different types of heavy metals and cadmium is just one of them. It would be better if we can test other heavy metals as well in order to assess the efficiency of removing other heavy metals by couch grass and arbuscular mycorrhiza fungi.
3. What is the condition for the arbuscular mycorrhiza fungi and couch grass to remove the highest percentage of cadmium in soil?

   - In this experiment, we set particular temperature, humidity, water amount and light intensity. It would be best to know whether there are other conditions which can cause them to grow faster and remove cadmium more efficiently

6. Conclusion

From this experiment, we showed that infection rate would increase with concentration of Cd in soil and our newly invented technology, FunGsorb capsule, could be a potentially used for phytoremediation of heavy metal contaminated soil. By means of symbiotic association, arbuscular mycorrhiza fungi and couch grass can survive in this soil model. It is believed that arbuscular mycorrhizae fungi is playing an important role in the increment of couch grass growth and soil trapping capability of its root; in which, availability of nutrients from soil can be increased and provide for the grass growth. Besides, the increment of soil trapping capability of root can contribute for metal absorption and retention; that mean heavy metals, such as cadmium used in this soil model, can be more effectively removed from the soil while we harvesting the grass. In conclusion, the one-step technology would be a potential solution for decontamination of heavy metal contaminated soil.

7. Bibliography

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8 Appendix I: Infection rate of couch grass by arbuscular mycorrhizae fungi

Sample from Jordan Valley Park

Slide:1	Slide:2	Slide:3	Slide:4	Slide:5	Slide:6	Slide:7	Slide:8	Slide:9	Slide:10
0%	0%	25%	25%	50%	0%	0%	25%	25%	50%
Slide:11	Slide:12	Slide:13	Slide:14	Slide:15	Slide:16	Slide:17	Slide:18	Slide:19	Slide:20
0%	0%	50%	25%	25%	25%	0%	25%	0%	0%
Slide:21	Slide:22	Slide:23	Slide:24	Slide:25	Slide:26	Slide:27	Slide:28	Slide:29	Slide:30
0%	25%	0%	25%	25%	0%	0%	0%	0%	25%
Slide:31	Slide:32	Slide:33	Slide:34	Slide:35	Slide:36	Slide:37	Slide:38	Slide:39	Slide:40
0%	0%	0%	25%	0%	25%	25%	25%	0%	0%
Slide:41	Slide:42	Slide:43	Slide:44	Slide:45	Slide:46	Slide:47	Slide:48	Slide:49	Slide:50
25%	0%	0%	0%	25%	25%	25%	0%	0%	25%

Sample from Kowloon Park

Slide:1	Slide:2	Slide:3	Slide:4	Slide:5	Slide:6	Slide:7	Slide:8	Slide:9	Slide:10
25%	25%	25%	0%	0%	0%	0%	0%	0%	25%
Slide:11	Slide:12	Slide:13	Slide:14	Slide:15	Slide:16	Slide:17	Slide:18	Slide:19	Slide:20
0%	0%	0%	25%	25%	25%	25%	0%	25%	25%
Slide:21	Slide:22	Slide:23	Slide:24	Slide:25	Slide:26	Slide:27	Slide:28	Slide:29	Slide:30
25%	0%	25%	0%	0%	0%	25%	0%	0%	0%
Slide:31	Slide:32	Slide:33	Slide:34	Slide:35	Slide:36	Slide:37	Slide:38	Slide:39	Slide:40
25%	0%	25%	0%	0%	0%	0%	25%	0%	25%
Slide:41	Slide:42	Slide:43	Slide:44	Slide:45	Slide:46	Slide:47	Slide:48	Slide:49	Slide:50
25%	25%	0%	25%	25%	0%	25%	25%	0%	0%

Sample from Yung Yuen Road, Yuen Long

Slide:1	Slide:2	Slide:3	Slide:4	Slide:5	Slide:6	Slide:7	Slide:8	Slide:9	Slide:10
50%	50%	100%	50%	25%	0%	50%	0%	50%	100%
Slide:11	Slide:12	Slide:13	Slide:14	Slide:15	Slide:16	Slide:17	Slide:18	Slide:19	Slide:20
25%	0%	25%	25%	50%	50%	50%	25%	100%	100%
Slide:21	Slide:22	Slide:23	Slide:24	Slide:25	Slide:26	Slide:27	Slide:28	Slide:29	Slide:30
50%	100%	50%	50%	50%	100%	25%	25%	25%	25%
Slide:31	Slide:32	Slide:33	Slide:34	Slide:35	Slide:36	Slide:37	Slide:38	Slide:39	Slide:40
25%	50%	25%	25%	50%	50%	25%	50%	50%	25%
Slide:41	Slide:42	Slide:43	Slide:44	Slide:45	Slide:46	Slide:47	Slide:48	Slide:49	Slide:50
25%	25%	50%	100%	100%	100%	25%	25%	50%	50%

It was found that only groups added with mycorrhiza seeds have arbuscular mycorrhiza fungi infection:

Group I: Arbuscular mycorrhiza fungi seed + Cadmium + couch grass seed

Slide:1	Slide:2	Slide:3	Slide:4	Slide:5	Slide:6	Slide:7	Slide:8	Slide:9	Slide:10
100%	100%	100%	100%	75%	75%	75%	100%	75%	100%
Slide:11	Slide:12	Slide:13	Slide:14	Slide:15	Slide:16	Slide:17	Slide:18	Slide:19	Slide:20
75%	75%	75%	75%	75%	25%	100%	25%	50%	25%
Slide:21	Slide:22	Slide:23	Slide:24	Slide:25	Slide:26	Slide:27	Slide:28	Slide:29	Slide:30
0%	75%	50%	75%	75%	100%	75%	100%	100%	75%
Slide:31	Slide:32	Slide:33	Slide:34	Slide:35	Slide:36	Slide:37	Slide:38	Slide:39	Slide:40
100%	100%	100%	75%	100%	100%	100%	75%	100%	100%
Slide:41	Slide:42	Slide:43	Slide:44	Slide:45	Slide:46	Slide:47	Slide:48	Slide:49	Slide:50
100%	100%	100%	100%	75%	75%	75%	100%	50%	75%

Group II: Arbuscular mycorrhiza fungi seed + couch grass seed

Slide:1	Slide:2	Slide:3	Slide:4	Slide:5	Slide:6	Slide:7	Slide:8	Slide:9	Slide:10
25%	0%	0%	25%	50%	25%	75%	100%	100%	50%
Slide:11	Slide:12	Slide:13	Slide:14	Slide:15	Slide:16	Slide:17	Slide:18	Slide:19	Slide:20
0%	0%	100%	25%	50%	25%	75%	100%	75%	50%
Slide:21	Slide:22	Slide:23	Slide:24	Slide:25	Slide:26	Slide:27	Slide:28	Slide:29	Slide:30
100%	25%	50%	50%	0%	100%	0%	100%	100%	50%
Slide:31	Slide:32	Slide:33	Slide:34	Slide:35	Slide:36	Slide:37	Slide:38	Slide:39	Slide:40
100%	0%	25%	75%	25%	50%	25%	0%	50%	75%
Slide:41	Slide:42	Slide:43	Slide:44	Slide:45	Slide:46	Slide:47	Slide:48	Slide:49	Slide:50
25%	25%	50%	100%	75%	75%	75%	100%	100%	100%

Appendix II : The concentration of Cadmium in the soil collected by different field sites

Sample from field sites	Replciates	Cadmium (mg/kg)	Mean (s.d.)
Jordon Valley Park	1	1.138	0.4263
	2	0.163	0.4117
	3	0.230	
	4	0.174	
Kowloon Park	1	0.375	0.2470
	2	0.150	0.1106
	3	0.125	
	4	0.338	
Yung Yuen Road, Yuen Long	1	6.088	6.6848
	2	7.163	0.6235
	3	6.050	
	4	7.438	

Appendix III: The mass of plant (with soil trapped), mass of plant (without soil trapped) and mass of soil trapped by plant in the four

	Mass of plant (with soil trapped) (g)	Mass of plant (without soil trapped) (g)	Mass of soil trapped by plant (g)
Cadmium + couch grass seed + Arbuscular mycorrhiza fungi seed (Group I)	90.4	19.8	70.6
	115.23	37.7	77.53
	33.6	8.7	24.9
	77.2	33.2	44
couch grass seed + Arbuscular mycorrhiza fungi seed (Group II)	42.8	10.1	32.7
	44.322	3.6	40.722
	28.136	6.3	21.836
	27.9	3.4	24.5
Cadmium + couch grass seed (Group III)	3.462	2.3	1.162
	9.556	3.317	6.239
	7.920	1.437	6.483
	16.531	3.223	13.308
couch grass seed (Group IV)	5.19	0.96	4.23
	8.64	1.2	7.44
	9.43	1.06	8.37
	8.78	1.04	7.74

Appendix II: Chlorophyll test on the 4 groups

	Mass of leaves used (g)	Absorbance at 663nm	Absorbance at 645 nm	Con. of Chlorophyll a ($mgmL^{-1}$)	Con. of Chlorophyll b ($mgmL^{-1}$)	Con. of Chlorophyll a + b ($mgmL^{-1}$)	Con. of chlorophyll a + b per g of leave ($mgmL^{-1}g^{-1}$)
Cadmium + couch grass seed + Arbuscular mycorrhiza fungi seed (Group I)	2	0.658	0.512	6.98	8.65	15.63	7.82
	2	0.649	0.523	6.84	8.94	15.78	7.89
	2	0.653	0.504	6.94	8.49	15.43	7.72
	2	0.655	0.495	6.99	8.27	15.26	7.63
couch grass seed + Arbuscular mycorrhiza fungi seed (Group II)	2	0.245	0.228	2.50	4.07	6.57	3.29
	2	0.254	0.219	2.64	3.83	6.47	3.24
	2	0.249	0.208	2.60	3.60	6.20	3.10
	2	0.210	0.245	2.01	4.63	6.64	3.32
Cadmium + couch grass seed (Group III)	1.5	0.14	0.10	1.51	1.63	3.14	2.09
	2	0.182	0.13	1.96	2.13	4.09	2.05
	0.8	0.074	0.049	0.81	0.78	1.59	1.99
	2	0.185	0.15	1.95	2.57	4.52	2.26
couch grass seed (Group IV)	0.4	0.054	0.051	0.55	0.91	1.46	3.65
	0.8	0.102	0.09	1.05	1.58	2.63	3.29
	0.3	0.054	0.05	0.55	0.89	1.44	4.80
	0.2	0.051	0.049	0.52	0.88	1.40	7.00

Appendix IV: The concentration of Cadmium and other essential elements (N, P, K) in the soil trapped by couch grass before and after experiment

Group	Replicate	Soil trapped by root															
		Before experiment								After experiment							
		Total N(%)	Mean (s.d.)	Total P(%)	Mean (s.d.)	Total K(%)	Mean (s.d.)	Cadmium (mg/kg)	Mean (s.d.)	Total N(%)	Mean (s.d.)	Total P(%)	Mean (s.d.)	Total K(%)	Mean (s.d.)	Cadmium (mg/kg)	Mean (s.d.)
Cadmium + couch grass seed + Arbuscular mycorrhiza fungi seed	1	0	0	0	0	0	0	0	0	1.58	1.52	1.95	2.02	1.74	1.69	26.54	33.71
	2	0	0	0	0	0	0	0	0	1.62	0.26	1.94	0.1	1.68	0.11	28.45	8.02
	3	0		0		0		0		1.74		2.03		1.54		35.62	
	4	0		0		0		0		1.15		2.15		1.78		44.21	
couch grass seed + Arbuscular mycorrhiza fungi seed	1	0	0	0	0	0	0	0	0	1.14	1.21	1.89	1.87	1.85	1.9	1.86	2.21
	2	0	0	0	0	0	0	0	0	1.15	0.09	1.96	0.09	1.91	0.04	1.48	0.66
	3	0		0		0		0		1.26		1.74		1.87		2.54	
	4	0		0		0		0		1.32		1.87		1.95		2.94	
Cadmium + couch grass seed	1	0	0	0	0	0	0	0	0	1.06	1.1	1.05	1.09	1.52	1.72	15.84	15.49
	2	0	0	0	0	0	0	0	0	1.12	0.09	0.96	0.12	1.74	0.14	18.52	3.04
	3	0		0		0		0		1.002		1.11		1.86		11.28	
	4	0		0		0		0		1.21		1.24		1.77		16.33	
couch grass seed	1	0	0	0	0	0	0	0	0	1.08	1.05	1.21	1.15	1.52	1.71	0.654	0.88
	2	0	0	0	0	0	0	0	0	1.01	0.06	1.15	0.1	1.87	0.15	0.658	0.44
	3	0		0		0		0		0.99		1.01		1.77		1.541	
	4	0		0		0		0		1.11		1.24		1.68		0.658	

Appendix V: The concentration of Cadmium and other essential elements (N, P, K) in the soil not trapped by couch grass before and after experiment

Group	Replicate	Bulk soil															
		Soil not trapped by root								After experiment							
		Total N(%)	Mean (s.d.)	Total P(%)	Mean (s.d.)	Total K(%)	Mean (s.d.)	Cadmium (mg/kg)	Mean (s.d.)	Total N(%)	Mean (s.d.)	Total P(%)	Mean (s.d.)	Total K(%)	Mean (s.d.)	Cadmium (mg/kg)	Mean (s.d.)
cadmium + couch grass seed + Arbuscular mycorrhiza fungi seed	1	1.05	1.07	1.23	1.24	1.85	1.78	10.245	11.86	1.01	1	0.564	0.56	1.56	1.4	8.521	7.68
	2	1.14	0.07	1.35	0.09	1.82	0.08	9.811	2.76	1.03	0.06	0.521	0.04	1.52	0.17	8.444	0.97
	3	1.09		1.14		1.75		11.541		1.04		0.614		1.21		6.521	
	4	0.98		1.25		1.68		15.852		0.91		0.552		1.32		7.221	
couch grass seed + Arbuscular mycorrhiza fungi seed	1	1.05	1.04	1.32	1.26	1.81	1.7525	0.221	0.78	0.94	0.9	0.642	0.57	1.52	1.41	0.0541	0.05
	2	1.06	0.08	1.08	0.2	1.75	0.05	0.965	0.44	0.85	0.04	0.618	0.07	1.57	0.24	0.0414	0.01
	3	0.93		1.52		1.68		0.664		0.88		0.523		1.51		0.0551	
	4	1.12		1.12		1.77		1.254		0.92		0.501		1.05		0.0299	
cadmium + couch grass seed	1	0.98	1.01	1.04	1.2	1.54	1.62	11.742	12.03	0.96	0.91	0.921	0.91	1.04	1.09	10.32	9.63
	2	1.05	0.03	1.12	0.14	1.65	0.07	15.111	2.13	0.95	0.07	0.834	0.06	1.12	0.04	10.19	0.99
	3	1.02		1.32		1.58		10.858		0.91		0.947		1.13		9.84	
	4	0.99		1.31		1.69		10.415		0.81		0.954		1.08		8.17	
couch grass seed	1	1.04	1.03	1.28	1.27	1.74	1.66	0.778	0.97	0.98	0.97	0.941	0.93	1.24	1.3	0.415	0.39
	2	1.05	0.05	1.34	0.05	1.71	0.08	0.881	0.4	0.94	0.02	0.925	0.01	1.22	0.07	0.221	0.14
	3	1.08		1.22		1.62		1.548		0.99		0.944		1.35		0.358	
	4	0.96		1.24		1.58		0.668		0.95		0.921		1.36		0.549	