Title	Effect of Phaeocystis sp. Sprayed over deteriorated soil; a possible method which restores and fertilizes eroding barren land
Author(s)	Liang, Ning
Citation	高知工科大学, 博士論文.
Date of issue	2006-03
URL	http://hdl.handle.net/10173/359
Rights	
Text version	author

Effect of *Phaeocystis* sp. sprayed over deteriorated soil; a possible method which restores and fertilizes eroding barren land

Ning Liang

A dissertation submitted to
Kochi University of Technology
in partial fulfillment of the requirements
for the degree of

Doctor of Philosophy

Special Course for International Students
Department of Engineering
Graduate School of Engineering
Kochi University of Technology
Kochi, Japan

March 2006

Abstract

One of the main and direct causes of soil erosion is removal of surface materials by wind and/or water, which include inorganic soil particles, silt, organic matters and soil microbes. Because these surface materials are essential for vegetation, such removal of surface materials leads directly to land deterioration, which negatively impacts not only on natural environment, but on agricultural production and peoples' life as well.

Phaeocystis sp. is a unicellular marine alga encapsulated with a sticky and thick envelope, which has been proved to be a kind of polysaccharide. The cell is about 4-5μm in diameter with the polysaccharide capsule of 10-20μm in thickness. A preceding observation showed that when pouring water on the Takasu-soil in pots, the infiltrated water was almost clear if *Phaeocystis* sp. suspension was sprayed *a priori*, while the water was turbid if only water had been given.

An attempt was thus made to study the effects of the marine alga sprayed on the soil on reduction of soil loss and improvement of soil and land conditions. Two modes of experiments were conducted in this study. The one is the primary, physical effect of alga spray on the amount of silt which contaminates in the water flowing from the soil surface and infiltrating through soil layer. The other is the secondary, indirect or long-term effect of alga sprayed on chemical and biological properties of the soil. Two kinds of experimental procedures were employed; 1) column experiment: soil sample was packed in a column to measure mostly vertical processes / changes in the soil before and after alga spray and 2) box experiment: soil samples was packed in a box to measure mostly horizontal processes/changes in/on the soil before and after alga spray. The changes in the soil were measured by OD_{600} as the amount of silt, OD_{420} as the amount of alga (chlorophyll), total organic carbon (TOC) and total saccharide (TSC) for estimation of humus etc., and ATP and respiration for estimation of living microbes in the soil. Four kinds of soil differing in texture were tested.

The results of column experiment showed that algae application reduced the amount of silt in the outflow from the bottom of the column while the algae were located on the top of the soil. In box experiment, the algae spray also reduced silt loss in runoff from soil surface of all kinds of soil tested.. Both ATP and respiration 4 weeks after algae sprayed increased especially in less nutrient soil.

The results clearly suggest that *Phaeocystis* sp. sprayed on soil can reduce silt flow and increase the activity of soil microbes. Even on limited types of the soil texture used in this study, promising recovery of eroded soil is shown by spraying the suspension of *Phaeocystis* sp. on the soil. Especially, the alga effect was more intensified when applied on sandy loam than silty /clay soil. The Loess plateau in western China would be vegetated by unicellular marine algae, which can be cultured with use of salt water from salt lakes in Qing Zang Plateau.

CONTENTS

Ⅰ Introduction

I - 1 Land deterioration and soil erosion

Land deterioration is one of the most serious environmental problems threatening peoples' life and social development. Soil erosion, a main cause of the land deterioration, is defined as removal of surface materials by wind or water, which includes inorganic soil grains, organic matters, soil microbes and vegetations. Soil under the erosive condition loses its physical texture and structure, and its nutrition to vegetations. The fine particles of organic matters, silt and clay are the life essence of the soil, mostly supplying nitrogen, potassium and phosphorus for plants. Furthermore, deposit of off-site soil in rivers and reservoirs affects water quality and becomes pollution source to water bodies nearby. Susceptibility and the rate of erosion depend on geology, geomorphology, climate, soil texture, soil structure, type and density of vegetation on land and land usage. Natural soil erosion usually does not lead to land deterioration, but erosion can be intensified by human immoderate activities and finally results in land deterioration. Soil erosion induced by rain splash is classified to sheet erosion, rill erosion and gully erosion. Sheet erosion takes place through detachment of a uniform thin layer of soil containing nutrition, organic matters and microbes by raindrop splash or run-off water. Rill erosion often occurs with sheet erosion, identified as a series of little channels or rills up to some tens cm deep. When rills continue to lose much larger volumes of soil, they are referred to gully erosion. Rill and gully erosion is the dominant form of water erosion in many parts of the world. Soils generally increase their erodibility as the particle size decreases, and are particularly susceptible to erosion under less vegetative and heavy rainfall conditions in summer. Raindrops displace soil particles and destroy soil structure of exposed soil and become a predominant initiating factor of soil erosion. Total annual soil loss in the world was almost 77 billion tons, corresponding to the soil flow of 571t $km^{-2}year^{-1}$. The annual soil loss in Asia was 27 billon tons corresponding to the soil flow of 611t $km^{-2}year^{-1}$ (Zachar, D. 1982). The latest remote-sensing survey shows that China has 3.56 million km^2 of soil erosion areas, accounting for 37 percent of the total territory of P. R. China. Around 3 billion tons of silts are being carried away annually from the catchment areas of the Yellow River and the Yangtze River, influencing 28% of the territory of China. Table 1 shows a comparison of turbidity of great rivers in the world.

Table 1. Turbidity comparison of 4 great rivers in the world (Zachar, D. 1982).

	Nile	Amu Darya	Colorado river	Yellow river
Turbidity (kgm^{-3})	1	4	10	40

In some cases, when soil is sterilized to ensure crop production, useful microbes and their hypha adhering soil particles are also eliminated. The soil becomes disaggregated and more vulnerable to splashing and run-off water. This is the case in Takasu village in Gifu prefecture in Japan. Takasu village is located in highland at the origin of the Nagara River, and is famous for its radish. Because of fumigation of soil by chloropicrin every year for better quality of radishes, the soil lost aggregates, and soil particles became easy to be washed down to the Nagara River. The silt sediment covered moss at the bottom of the River, on which its famous ayu-fish is living on. The research institute of Kochi University of Technology had been asked to develop a method for reduction of silt flow to the Nagara River.

I - 2 Treatment of soil with polyacrylamide and others

Soil erosion control may be achieved through enhancement of infiltration capacity of the soil and reduction of runoff volume, improvement of the soil aggregate stability and its resistance to erosion, increasing roughness of ground surface and reducing velocity of runoff and wind, prevention of the soil from raindrop splash. Accordingly, soil conservation practices include agronomic measures (covering soil surface, increasing surface roughness, surface depression storage and infiltration), soil management methods (fertilizers, manures, and drainage), and mechanical measures (contouring, ridging, terraces, shelterbelts and water ways). Polyfunctional polymers have been utilized as soil conditioners. One of the soil management methods is stabilization of soil temporarily from 2 weeks to 6 months. The polymers develop chemical interaction with the clay mineral in the soil and assist to form soil aggregates, which increases size and stability of the soil pore and raises the infiltration rate. (Kirkby, M. J. and Morgan, R. P. C. 1981). Anionic polyacrylamide (PAM) is synthesized out of natural gas associated with petroleum production. Studies showed that PAM applied in furrow and sprinkler irrigation increased final infiltration rates and reduced runoff during simulated rainfall. (Smith et al., 1990; Levin et.al., 1991; Levy and Agassi, 1995; Ben-Hur and Keren, 1997). Lentz and Sojka (1994) reported that PAM reduced soil loss by 93% under certain application strategies (10g m^{-3} during irrigation water furrow advance). However, because PAM is likely to contain a cancer inducer monopolyacrylimide, and natural gas is a limited resource, it is necessary to seek the environmentally friendly and sustainable resource for soil conditioners. The earlier research on erosion control showed that polysaccharides and cheese whey could be substitute to PAM (Brown et al., 1998; Shainberg and Levy, 1994). Some starch xanthate, microfibril suspension and chitosan-based polymer also showed effectiveness on reduction of soil loss. These biopolymers can replace PAM, enhance biodegradability and benefit to environment (Orts et al., 1999, 2000, 2001, 2002).

I - 3 *Phaeocystis* sp.

Phaeocystis sp. is a unicellular marine alga and classified as a member of the class *Haptophaceae* / *Prymnesiophyceae*. The taxonomy of this genus / species remains unclear so far. The alga is encapsulated with a thick mucilaginous envelope. Massive accumulation of this gelatinous capsule sometimes clogs fishing nets and fish gills to be choked. When the alga was treated with acid or heat, the capsule could be separated from the cell. Laboratory analysis has proved that the mucilaginous capsule is a form of extracellular polysaccharide, and was named haptose after *haptophaceae*. The haptose powder consists of about 40% saccharide, 35% ash, 10% water, 4% protein and 4% lipid; the apparent molecular weight of haptose was determined about 4,000, 000 by gel filteration chromatography; haptose was composed of glucose and xylose at ratio of 1: 1 (Yamashita 2002). Studies also showed that haptose can be used as principal component of a water clarifier “Mizusumashi” (Seki, 2004), as a raw material of biodegradable mulch (Morikawa, 2001), as a component of the plantation pile (Kurachi, 2003) and biodegradable film (Okahana, 2002). Because of the biochemical composition and mucilage property of its extracellular polysaccharide capsule, *Phaeocystis* sp. is biodegradable and could supply nutrition for soil microorganisms, and would improve biological activity of the soil. The mucilage material in extracellular polysaccharide can facilitate to form soil aggregates. Previous observations on spraying *Phaeocystis* sp. suspension on soil pots (Fig. 1) have indicated that infiltrated water from the bottom of the pots was almost clear while the water from the water sprayed pots appeared turbid. Soil treatments by alga suspension are thus promising but similar experiments have not been reported so far.

Fig. 1. Pictures of preliminary pots test.   Soil: Takasu soil 5kg.

Left pot: water 2L sprayed   Right pot: algae suspension 50g/2L sprayed

I - 4 What is to be investigated

An ecosystem includes living organisms and non-living substances interacting mutually and exchanging materials (Odum, 1959). Soil in natural ecosystems has intrinsic physical, chemical and biological succession processes, and maintains certain level of homeostatic equilibrium. In managed natural ecosystems where neither erosion nor percolation occurs, a balance exists between matter input and output. When the equilibrium is disturbed by external force, changes take place in soil physical property, i.e. moisture, stability and structure, in soil chemical property, i.e. fertility and pH, C, N, P, and in soil biological property such as microbial biomass. Therefore, experiments in this research were designed in physical, chemical and biological points of view as following:

(1) Column experiment: To study the effect of *Phaeocystis* sp. spray on reduction of soil loss from the subsurface; to identify the vertical distribution of silt and the location of *Phaeocystis* sp.; to study correlation of compactness, alga concentrations, rainfall intensity and silt loss.
(2) Box experiment: To study physical, chemical and biological effects of *Phaeocystis* sp. suspension spray on soil surface; to compare the effect of several suspension application rates and identify optimum application rates in three types of soil.

I - 5 What has been found

Soil loss from the surface of the boxes and from the bottom of the columns has been significantly reduced in *Phaeocystis* sp. spray. Total organic carbon and total saccharide in treated soil did not increase significantly in relatively rich nutrient soil, but increased in poor nutrient soil; soil biological activity expressed by the amount of soil ATP and respiration rate in eroded barren land increased greatly. It was found in this study that *Phaeocystis* sp. when sprayed in suspension on land, it will work for preventing silt from flow out and supporting soil microbes to grow.

Ⅱ Materials

Ⅱ - 1 Soil

Ta: Takasu soil was topsoil of high-land radish farm, which was fumigated with chloropicrin annually, in Takasu village, Gifu prefecture, Japan. Ta soil was shifted with a 6.4mm screen and was kept in plastic bags at room temperature and moisture.

Ka: Kanuma soil (small size, < 5mm) was purchased from a gardening shop.

Aka: Akatama soil (small size) was purchased from a gardening shop.

Kah: Kahoku soil was obtained from a farm land in Kahoku town and was shifted with a 6.4mm screen.

TK: A mixture of Takasu soil and Kanuma soil (1:1). Kanuma soil was sprayed water and adjusted water content similar as Takasu soil and mixed with Takasu soil and the mixture was kept in plastic bags in room temperature.

KAK: A mixture of Kanuma soil, Akatama soil and Kahoku soil (3:1:0.5). Kanuma soil, Akatama soil and Kahoku soil (air dried) were shifted with a 2.5mm screen separately and then mixed them together at the given weight ratio and kept in a storage container at the room temperature and moisture.

Soil textures of 4 soils, determined by the hydrometer method (Garrett, S. D. 1981.) are shown in Table 2. Soil compositions and types are shown clearly the USDA textural triangle in the Fig. 2.

Table 2. Composition of Ta soil, TK soil (1:1), Ka soil, KAK soil (3:1:0.5)

	Clay (%)	Silt (%)	Coarse sand (%)	Fine sand (%)
Ta soil	54	32	5	9
TK soil	40	31	19	10
Ka soil	5	26	41	28
KAK soil	12	26	45	17

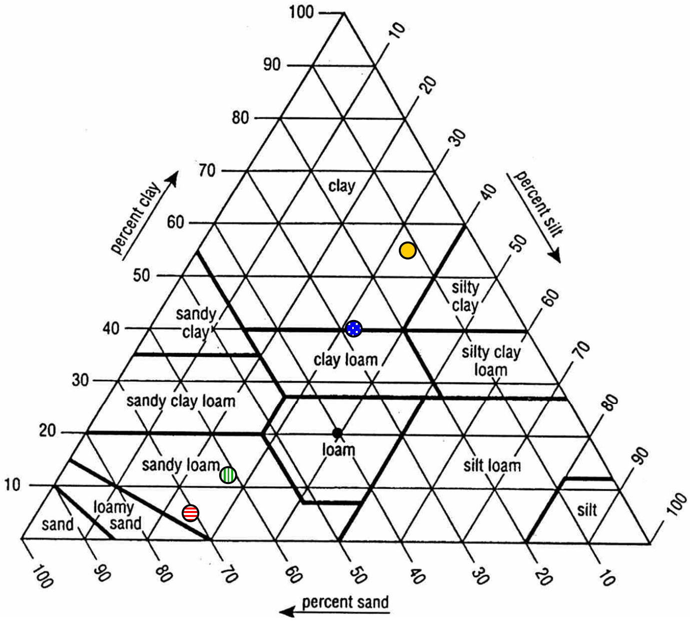


Fig. 2. Four soil compositions are described in the USDA textural triangle
○ Takasu soil; ○ TK: a mixture of Takasu and Kanuma soil (1:1);
○ KAK: a mixture of Kanuma, Akatama, and Kahoku soil (3:1:0.5) ○ Kanuma soil

Ⅱ - 2 Algae

Phaeocystis sp. is of *haptophacae* (subclass *Prymnesiophycidae*); a unicellular marine alga encapsulated with a mucilaginous envelope of polysaccharide. Under an optical microscope, the polysaccharide layer which surrounds the cell body and excludes the “sumi” ink in the background can be seen (Fig. 3). The cell size is about 4-5μm, with a polysaccharide layer of 10-20μm thick. The dry mass ratio is about 1:1 for cell and polysaccharide. *Phaeocystis* sp. is a kind of algae that causes red tide. The strain was collected in Okinawa, Japan, and isolated and cultured in the laboratory.

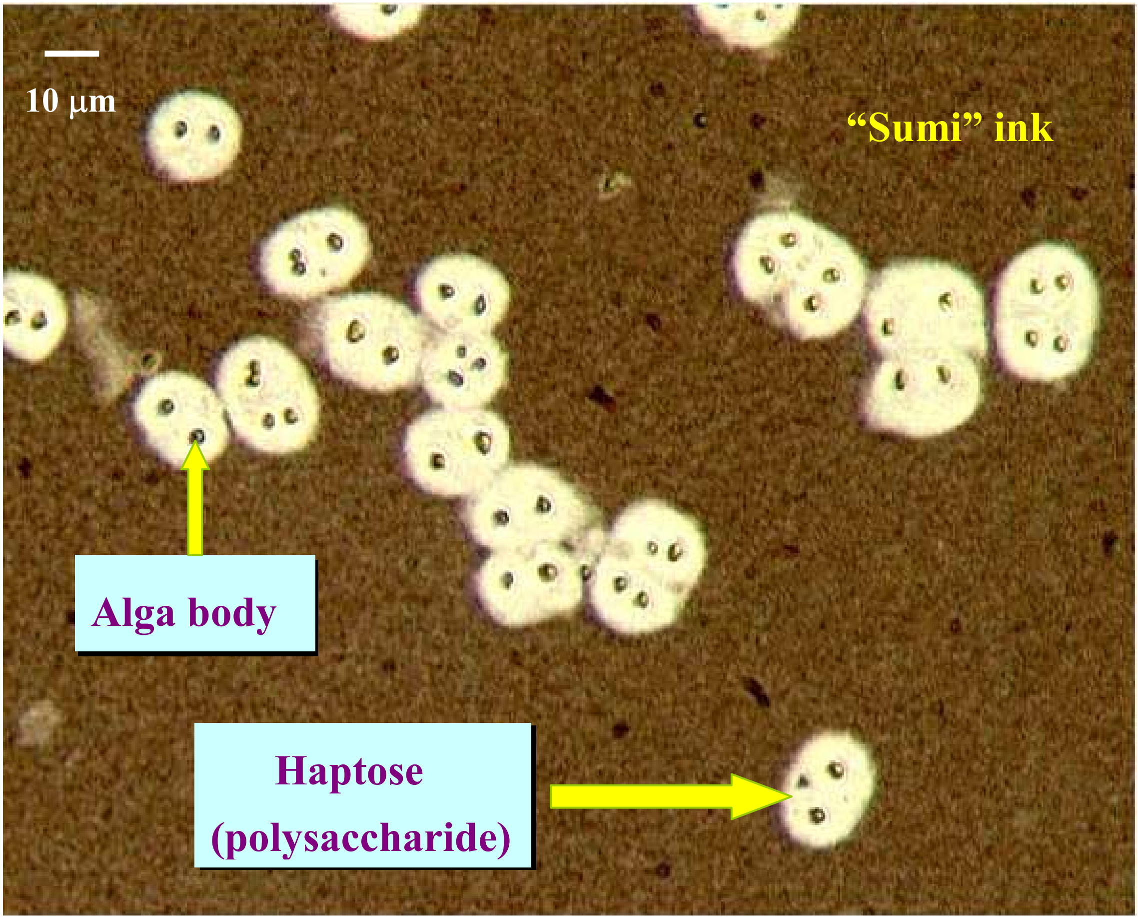


Fig. 3. Picture of *Phaeocystis* sp. under microscope

Phaeocystis sp. was cultured in artificial sea water (Akuamarin®) with an enriched reagent (PES) mixture. Firstly, *Phaeocystis* sp. was inoculated on 1.5% agar PES medium plate, and then cultured for 16h in the light (average illumination about 3,000 Lux) and in the dark 8h at 25℃. Brown colonies appeared after 1-2 weeks and the colonies were transferred into liquid medium independently in test tubes, 50ml conical flasks then 500ml conical flasks to enlarge culturing scale under shaking and illumination (16L-8D) at 25℃. Four weeks later, the liquid culture was mixed with PES enriched sea water in a 100L transparent plastic tank, illuminated from the side by fluorescent lamps (16L-8D), and aerated by an air compressor which supplies air bubbles (20L/min) not only to send CO_2 but also to stir the liquid medium for 4 weeks culturing (Fig. 4). The *Phaeocystis* sp. was collected by a continuous filtering system, then centrifuged at 4000*g for 20min. Gelatinous pellet of *Phaeocystis* sp. was put in polyethylene bottles and stocked in a refrigerator at 4℃.

Fig. 4. Picture of *Phaeocystis* sp. culturing in 100L tank
Left tank: younger culture Right tank: older culture

In the following experiments / results, the amounts of *Phaeocystis* sp. was expressed in grams of wet pellet, one gram of which was equivalent to about 30mg of dry alga. A half of the dry alga was of cell body and the other half was of polysaccharide in weight.

Phaeocystis sp.(collected in May 24, Oct. 17, Nov15, 2004 and mixed together) which had been condensed to be gel and kept in refrigerator, was suspended with 30 times of water (w / V) and was centrifuged at 10,000rpm for 20min to make salt free.

Ⅲ Procedures

Ⅲ - 1 Column experiment

The main objective of this column experiment was to confirm the infiltration, and the vertical distribution of silt and algae. A polyvinyl chloride (PVC) water plumbing pipe (inner diameter 4.4cm) was cut to 30cm in length as column. A piece of 100mesh sheet was applied to block big particles flowed out and a bended connecter which acted as a funnel for outflow water and sediment into a collecting container (Fig. 5). To avoid segregation, the soil was stirred and mixed prior to pouring. 150g mixed soil was filled into each column and compacted to avoid considerable gaps between soil and the wall. *Phaeocystis* sp. was prepared at a given concentration and then a given volume was sprayed on the soil surface in the column in 5min. Outflow was collected with a beaker and measured at 600nm for the turbidity. After the entire outflow flowed out, one stick that fit to the column was used to push the soil column carefully out from PVC pipe. The soil column was cut in parallel to the surface to 7 layers (discs) at 1, 2, 3, 5, 7, 9 cm deep from the surface. Each disc was put in one container and ready for determination of silt and alga chlorophyll. Each treatment was replicated three times.

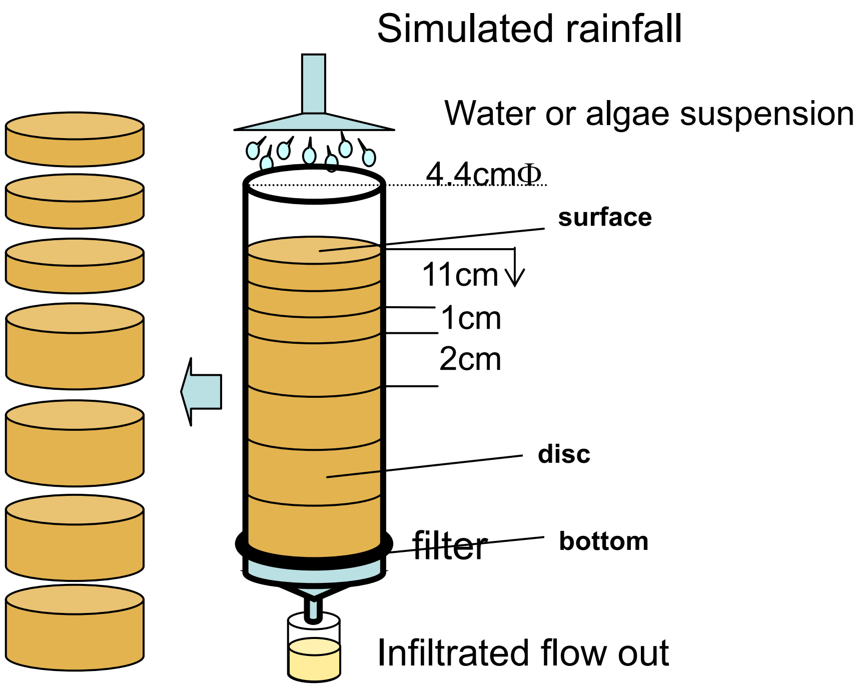


Fig. 5. Schematic picture of the column for outflow test and algae location experiment

Ⅲ-2 Box experiment

The objective of box experiment was to estimate the volume of runoff (surface flow from a slope) and the silt content in the runoff after *Phaeocystis* sp. was applied on the soil surface. Ta, TK and Ka soil were studied for the effects of algae on different soil. 27 boxes were made (Fig. 6). The box was 16 × 16 cm square and 25cm in depth. At one corner of the box, two holes of 1cm in diameter, at the bottom and at 15cm high from the bottom were drilled.

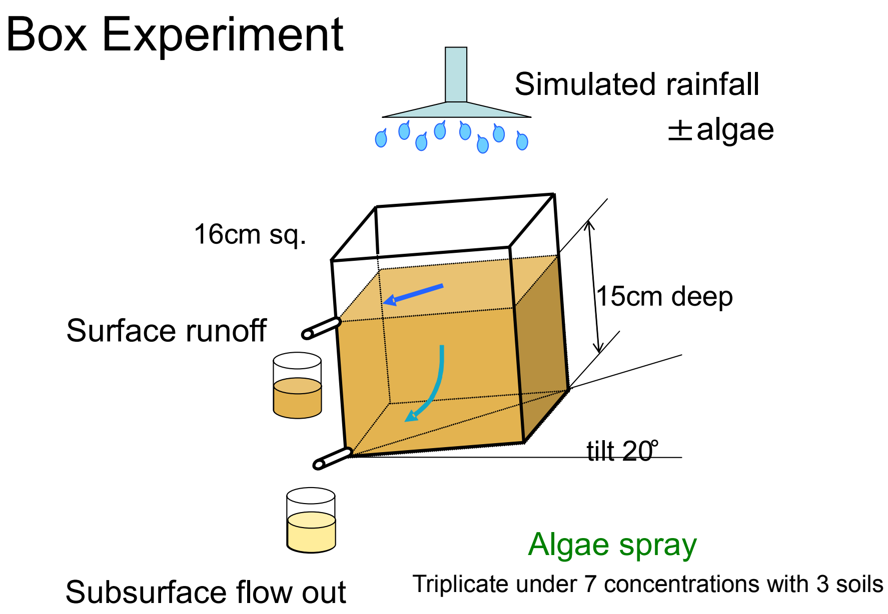


Fig. 6. The schematic picture of soil box experiment.

The soil was stirred and compacted prior to leveling to a uniform depth of 15 cm. The soil bulk density of Ta, TK, and Ka soil was about 1.6, 1.3 and 0.9. The soil boxes were put in ventilation, no sunshine place for days.

The surface flow (runoff) was collected and its volume was measured. The turbidity was measured by spectrophotometer at 600nm. After that, the runoff was filtered by 0.45 μm filter membrane, dried at 50℃ on the membrane and weighed for the silt amount.

A tube was attached on the top hole to funnel surface flow (runoff) into a container, while the bottom one covered with a piece of 100 mesh nylon screen to block the big particles flowing out, except for outflow with silt. The box was settled in a pan which was a container for the infiltrative outflow. The boxes were padded so surface slope could be varied. In this study, the slope was 20° .

In box experiment, each treatment was replicated three times; experiments were divided to three tests. Algae suspension was added in the first spraying in every test, followed by three water-only sprayings (simulated rainfalls). The interval was one week to allow the soil dried. Table 3 shows the procedures. After simulated rainfall was sprayed, the surface flow (runoff) and bottom flow (outflow) were collected and their volumes and OD_{600} were measured.

Table 3. The procedures of soil box experiment

Soil: Ta and TK						
	algae spray (150ml)			simulated rainfall (ml)		
Test	algae amount(g)			1	2	3
1	0	5	10	500	500	500
2	0	20	40	500	500	500
3	0	15	30	500	500	500
Soil: Ka						
	algae spray (150ml)			simulated rainfall (ml)		
Test	algae amount(g)			1	2	3
1	0	5	10	1000	1000	1000
2	0	20	40	1000	1000	1000
3	0	15	30	1000	1000	1000

Following the third rainfall spray of each test, soil samples were taken to a 1cm depth from the surface and sealed in plastic bags, and kept in the refrigerator at 4℃ for determining TOC, TSC, ATP and respiration measurements within 4 days.

Ⅲ - 3 Rainfall simulation

A sprayer was used to spray water / *Phaeocystis* sp. suspension in column experiments. The spraying speed was controlled at 150ml water/suspension in 5 min.

A watering can was used to spray water / *Phaeocystis* sp. suspension in box experiments. The spraying speed was controlled at 500ml water/suspension in 3 min.

Ⅲ - 4 Silt and OD_{600}

Optical density (OD) was measured by a spectrophotometer for estimation of the turbidity of outflow water. The actual weight of silt in outflow can be determined by filtrating, drying, and weighing.

Measuring turbidity was considered a simple and convenient method to replace measuring the weight of silt. After the correlation of residue in outflow and OD_{600} was found to be linear, OD_{600} could be used to indicate the silt amount under the condition of closed suspension volume. Or the linear relationship between OD_{600} and silt concentration was found, soil loss could be calculated. After algae suspension was sprayed on the soil surface, algae body could flow down along the soil gap. When SDS was used to extract chlorophyll of algae, the turbidity at OD_{600} of extracted suspension could be used to show the silt distribution in layers of soil column. Soil column was pushed out with caution and cut to be 7 discs; 5g soil was taken from each disc and mixed as possible. 3 % SDS solution 7ml was used to extract chlorophyll of *Phaeocystis* sp. which mixed with soil. After centrifuged at 3500rpm for 3min, the supernatant was measured at 600nm by spectrophotometer. Fig. 9 shows spectrograms of algae, soil with algae and soil being extracted by 3% SDS solution. From Fig. 9, OD_{600} was chosen to measure turbidity of soil with algae extracted by SDS.

Ⅲ - 4 - 1 Calibration of OD600 to silt concentration with KAK soil in column experiment and with Ta, TK and Ka soil in box experiment

Silt loss was used as a parameter of physical effect of the alga application on soil property in the thesis. In order to quickly detect the silt concentration during experiment course, correlation between OD_{600} and silt concentration should be established. After different volume of water was applied on soil columns, the outflow was collected and its OD was measured at 600nm. The suspension was filtered with a membrane filter of pore size 0.45μm and then the filter was dried at 50℃. The sediment on the filter was weighed. The correlation of OD_{600} and silt concentration for KAK soil is shown in Fig. 7. It is also noted that the calibration curves and equations are feasible only in column with closed volume of outflow. Real silt concentration test is necessary for the columns with large difference of volume of outflow.

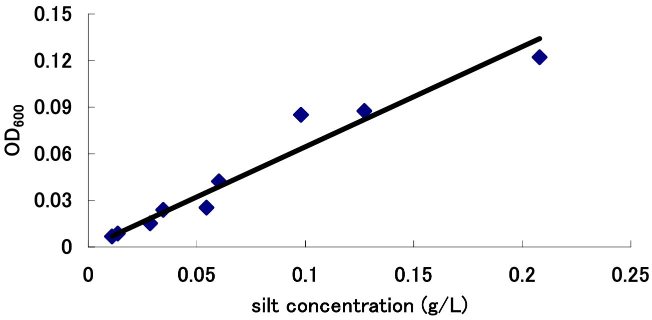


Fig. 7. Relationship between OD_{600} and silt concentration with KAK soil

In soil box experiment, when each water-only spray (simulated rainfall) was applied, each soil box's runoff from surface and outflow from bottom were collected. Ta, TK and Ka soil got 3 rates of relationships shown in Fig. 8A, Fig. 8B and Fig. 8C.

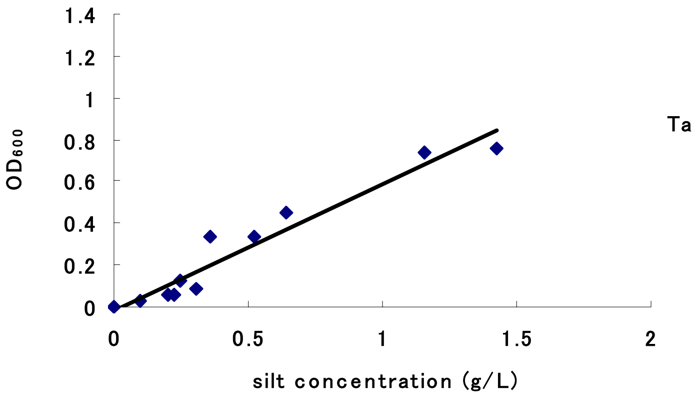


Fig. 8A

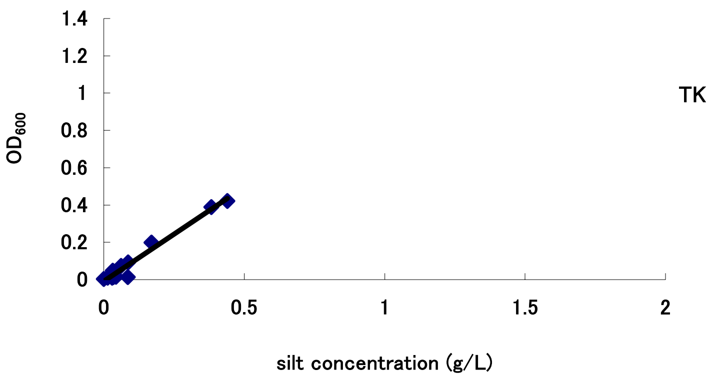


Fig. 8B

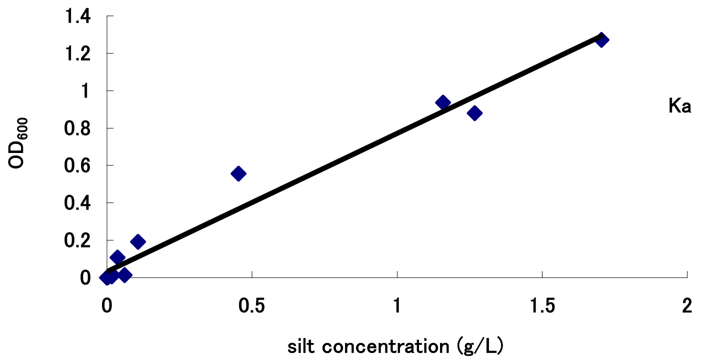


Fig. 8C

Fig. 8. Correlation of silt concentration and OD_{600} in runoff and outflow

A: Ta soil B: TK soil C: Ka soil

Ⅲ - 4 - 2 Silt loss from KAK soil treated with different soil compactness, alga content suspension, and rainfall amount.

1. 150g KAK air dried soil (<2.5mm) was filled into the column and packed for 0, 5, 10, 20, 40 times. 10mg/ml algae suspension 150ml (0.1g/cm^2) was sprayed in it and outflow was collected.

2. 150g KAK air dried soil (<2.5mm) was filled in the column and packed for 20 times, and then 0, 0.15, 0.375, 0.75, 1.5 and 3g algae suspended in 150ml water (0, 1, 2.5, 5, 10 and 20 mg/ml) were sprayed in the column and outflow was collected.

3. 150g KAK air dried soil (<2.5mm) was filled in the column and packed for 20

times, water and 20mg/ml algae suspension 150ml(0.2g/cm^2) was sprayed in the column and outflow was collected. After all the algae suspension flowed out, 0, 150ml, 50ml × 3 times, 150ml × 3 times of water (simulated rainfall) was sprayed in it. Each application was conducted after last application flowed out completely.

Ⅲ - 4 - 3 Distribution of silt in soil column after algae spray

Air dried 150g KAK soil (<2.5mm) was filled in the column and packed for 20 times, then 150ml each of water and a 20mg/ml alga suspension was sprayed in each column. From the soil column 5g of soil was taken from each disc and 7ml 3 % SDS solution was added to extract chlorophyll of *Phaeocystis* sp. in the soil. After centrifuged at 3,500rpm for 3min, the supernatant was measured at 600nm by spectrophotometer.

Ⅲ- 5 Distribution of algae; chlorophyll

Chlorophylls have been commonly used as semi quantitative estimation of algae (Vollenweider, 1968). Although chlorophyll can be extracted by acetone, ethanol or diethylether, acetone has been considered the best reagent to extract chlorophyll from plants and algae. After trials with acetone, ethanol, alkyldimethyl- benzylammonium chloride and sodium decylalkyl sulfate (SDS), 3% SDS was found to be the best reagent for extraction. 5g of sample soil was taken from each disc, placed in a 50ml centrifuged tube, mixed with 7ml of 3 % SDS solution and vortexed. After centrifuged at 10,000rpm for 20min, the supernatant was scanned by a spectrophotometer. Fig. 9 shows the absorption spectra of algae, soil with algae and soil. From the algae sprayed soil, the extraction efficiency with 3% SDS suspension was better than that with acetone. OD_{420} was thus chosen to determine chlorophyll of alga with soil.

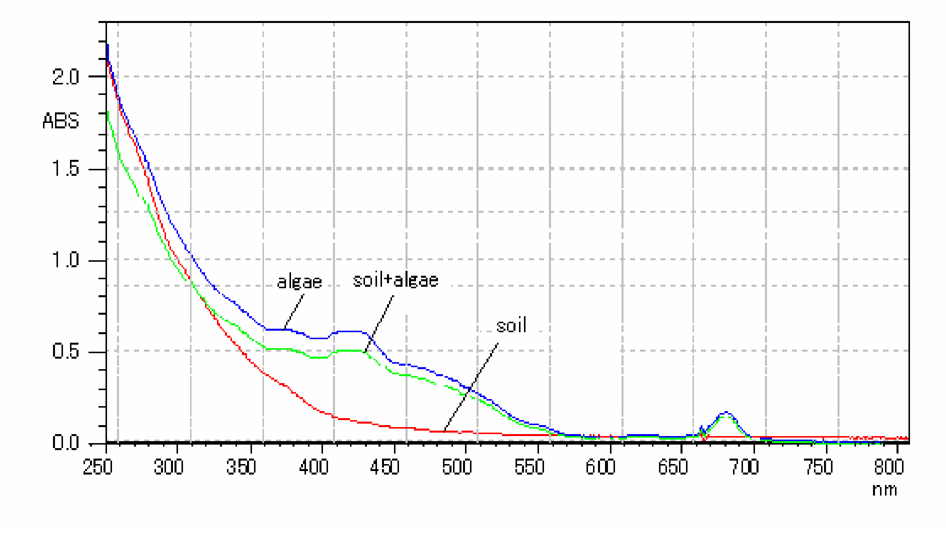


Fig. 9. Absorption spectra of algae, algae with soil and soil extracted by 3% SDS

Calibration for OD420 against KAK extracted by the SDS method is shown in Fig. 10.

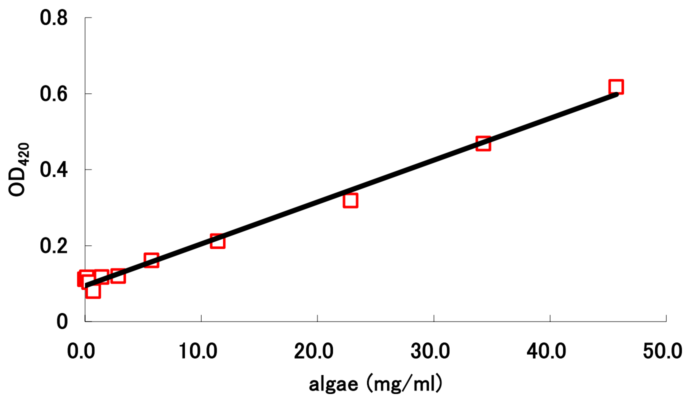


Fig. 10. Correlation of OD_{420} and algae with KAK soil extracted by 3% SDS solution

Ⅲ - 5 - 1 Distribution of alga in soil under the conditions of difference of soil compactness, algal concentration and the amount of rainfall.

The procedures were shown in Ⅲ - 4 - 2.

Ⅲ - 6 Total organic carbon (TOC)

Total organic carbon was measured by extensive oxidation of soil with dichromate and sulfuric acid. Indicator was ferroin. About 0.5g soil (containing 10-25mg of C) was put into a 100ml conical flask, 5ml each of 1/6M $K_2Cr_2O_7$ and conc. sulfuric acid were added and a funnel was put on the mouth of conical flask to reflex the steam. A group of flasks were placed on a preheated electric hot plate at 175 ±5 ℃ and made the mixture boil for 10min. After cooling the mixture, 4-5 drops of ferroin indicator was added and the mixture was titrated with 0.25M $FeSO_4$ to the end point of wine red mixture.

Measurement of algae addition with the dichromate-sulfuric acid method was carried out with 0.5g each of air dried KAK soil and given amounts of algae. The results are shown in Fig. 11.

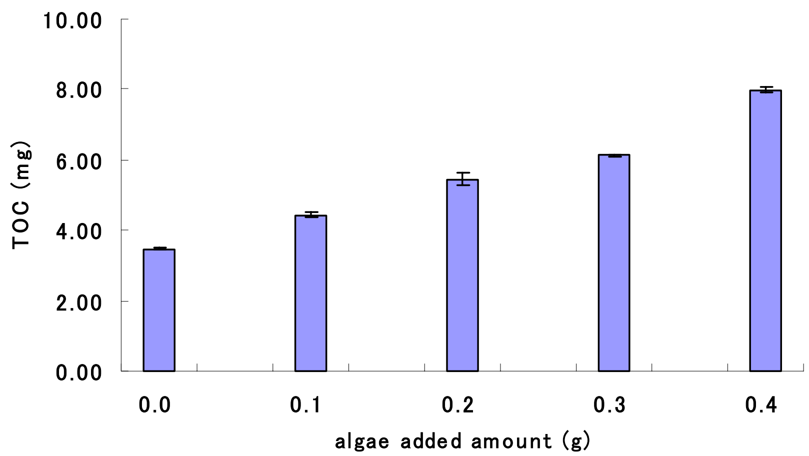


Fig. 11. TOC content of KAK Soil with algae additions. Bars represent standard errors of the mean.

Ⅲ - 7 Total saccharide (TSC)

Acid hydrolysis and phenol-sulfuric acid method was used to measure total saccharide which is expressed by glucose. 0.5g of soil (< 2.5 mm) and 5ml of 0.6M HCl were mixed in a tube then covered loosely with a cap. The tubes were placed in a high pressure steam sterilizer and heated at 121℃ for 20min. After the tube was cooled, the supernatant was taken out and adjusted to pH 7 then filled up to 6ml with distilled water.

The solution was diluted 5 times from which 200μl was mixed with 200μl of 5% phenol, then 1ml of concentrated sulfuric acid was injected rapidly. 30min later, OD_{490} was measured. The calibration curve was obtained with given amounts of glucose at each time when soil samples were determined. One result is shown in Fig. 12.

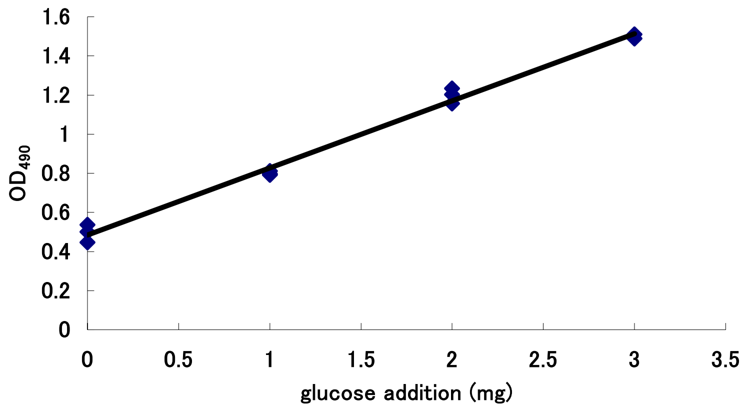


Fig. 12. Soil TSC working curve with glucose addition of 0, 1, 2, and 3mg

Ⅲ - 8 ATP

1g of the wet soil sample was thoroughly mixed with 1 ml dimethylsulfoxide (DMSO), and was vortexed for 30 seconds. This mixture was vortexed for 30s with each addition after 8ml of 0.01M of Na_3PO_4 was added in and then 2ml of TES-Mg^{2+} buffer (a mixture of 0.01M TES and 0.04M $MgSO_4$) at pH 7.50. 1ml of suspension was taken into a micro centrifuge tube and centrifuged at 12,000 rpm for 3min. 0.5ml supernatant was taken into a vial and was inserted into the luminescence measuring apparatus. 0.5ml of the lucifirin-lucifirase reagent (Nisui kit) mixed and diluted with TES-Mg^{2+} buffer was injected into the vial. Photons were integrated over a period of 10sec for 10 cycles. Calibration curve was obtained at the same time of soil sample measurement. 1g KAK soil (sterilized at 240℃ for 16h by dry heat sterilization) was mixed with 1ml of DMSO, 10ul each of a given concentrations of Adenosine 5'-triphosphate (ATP) were added into tubes and followed procedures as described for the samples. One calibration curve is shown in Fig. 13.

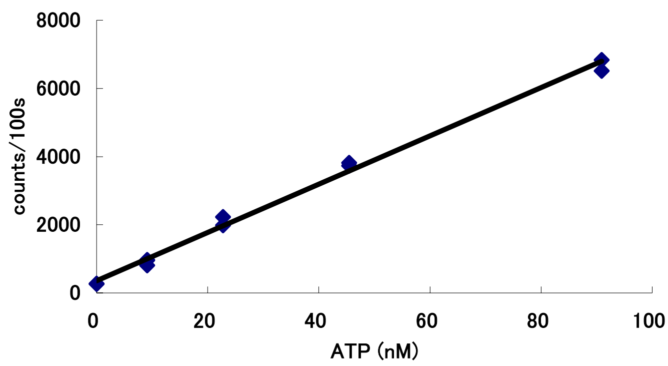


Fig. 13. A calibration curve for soil ATP

Ⅲ - 9 Respiration

The real-time soil respiration was measured with newly invented electronic micro – manometer. As shown in Fig. 14, the apparatus consists of two sealed sample boxes, a recorder and an electronic micro – manometer. Each box contains a jar with 2.5g soil and 5ml 4M KOH solution respectively. A fine tubing connects two jars through an electronic micro – manometer. The soil in one jar glucose solution was fed, while the soil in the other jar the same volume of water was added. If there is respiring activity, CO_2 will be evolved and adsorbed by KOH. Air pressure difference affects voltage output, which can be recorded. All parts of apparatus except the recorder are put in a big cellular polyethylene box with a cover to decrease the effect of temperature.

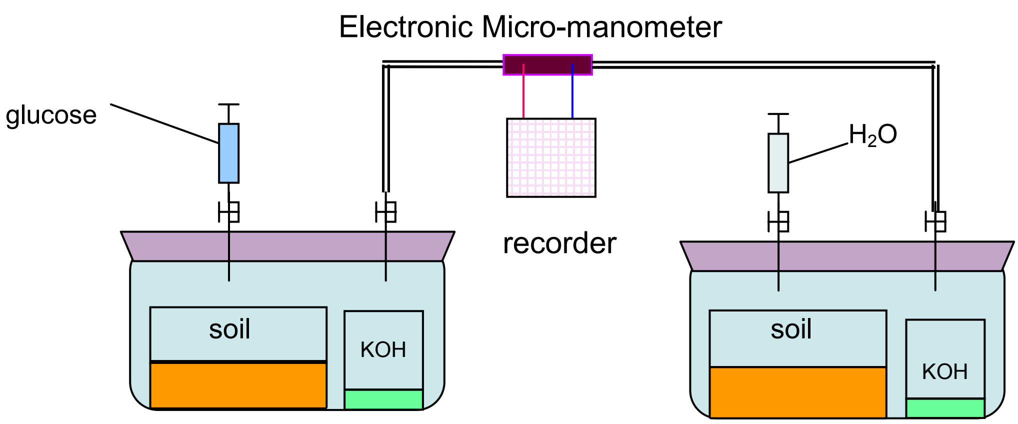


Fig. 14. Schematic picture of a measuring system for soil respiration

Calibration was carried out with given amounts of Kah soil (Fig. 15)

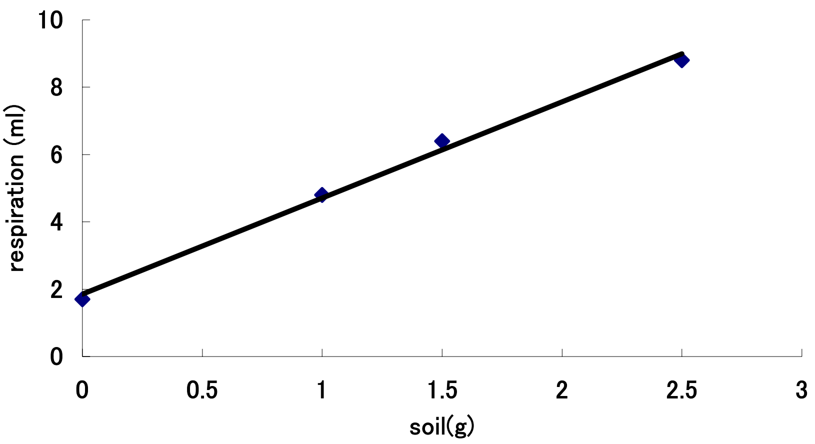


Fig. 15 soil respiration calibration curve

Ⅳ Results

Ⅳ - 1 Column experiment

Ⅳ - 1 - 1 Soil loss through a soil column.

Silt concentration decreased when soil compactness increased by increasing times of packing soil (Fig. 16). However, differences of silt concentration between packing 5 and 10 times, and between 20 and 40 times were not obviously different. Packing soil for 20 times was chosen in other column experiments.

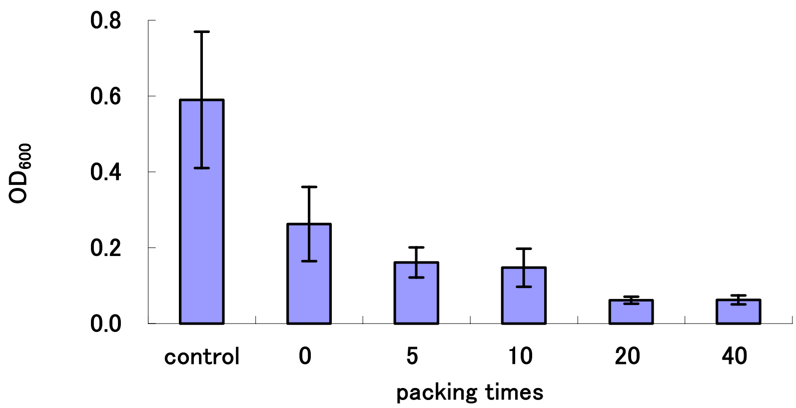


Fig. 16 Variety of outflow turbidity with packed times of KAK soil column. Control means 150ml water sprayed. 0 means the soil was simply filled in the column. Algae suspension 10mg/ml 150ml was sprayed in columns packed for 0 – 40 times.

Silt concentrations of outflow from all the columns after alga spray were lower than the control (Fig. 17). The results suggest that the alga content of 10mg/ml on 15cm^2 might be effective to reduce silt loss.

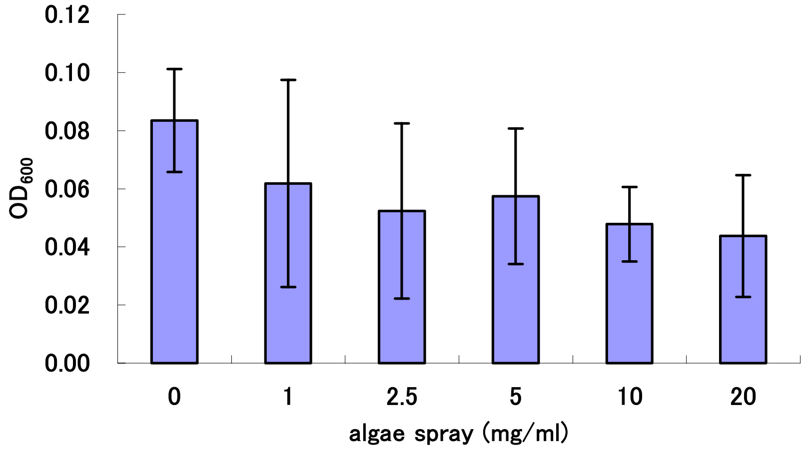
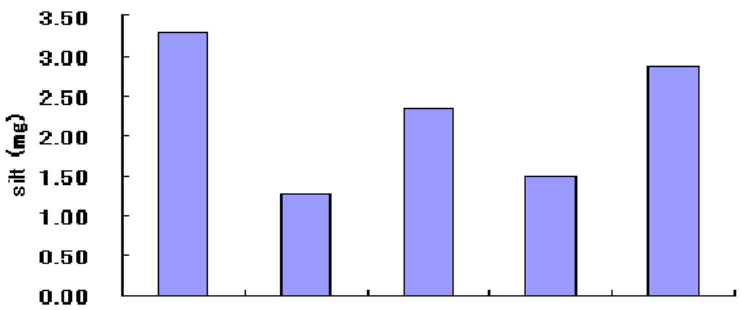


Fig. 17. Outflow turbidity from KAK columns after alga spray at different concentrations

Under different sizes of simulated rainfall, the silt concentrations from the KAK soil columns were also lower in the alga sprayed columns than the control (Fig.18). The silt concentrations in the non rainfall column (2) and in the treatment of 50ml rainfall 3 times (4) appeared similar. However, when the same amount of rainfall were given in the column 3 and 4, the silt in the column 3 and 4 were obviously different, which reveals that weak rainfall leads to less silt loss.



column	1	2	3	4	5
2% algae(ml)		150	150	150	150
water(ml)	150		150	50 × 3	150 × 3

Fig. 18 Soil loss from the bottom of KAK soil column with simulated rainfall following algae application

Ⅳ - 1 - 2 Algae distribution in a soil column

The chlorophyll concentration in each soil disc represents the vertical distribution of sprayed algae. The chlorophyll was found mainly on the top disc of the column and only a small amount of algae flow down the column (Fig. 19a). Fig. 19b showed that turbidity of the SDS extraction supernatant of soil on the top disc of the column was clearly lower than other discs. The result revealed that the sprayed alga mainly stayed on the top layer of the soil.

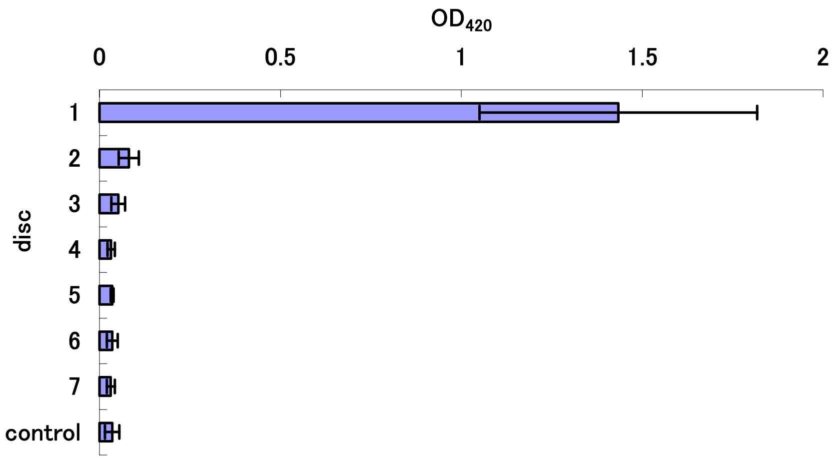


Fig. 19a. Distribution of sprayed alga (OD_{420}) in the soil discs after 20 mg/ml algae suspension (150ml) (0.2g/cm^2) sprayed on the column. Bars represent standard errors of the mean.

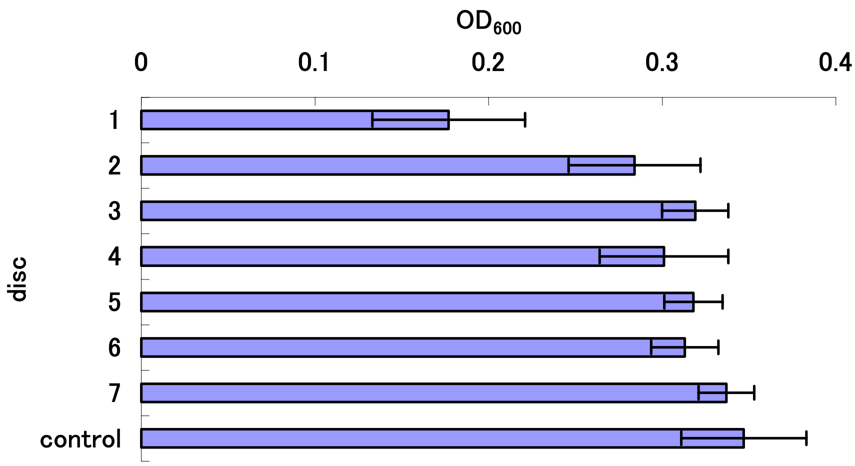


Fig. 19b. Turbidity of the suspension extracted from each soil disc with 3% SDS after spraying 20mg/ml algae suspension (150ml) on the column. Bars represent standard errors of the mean.

When alga was applied at various amount in the same volume, it showed again that the alga mostly stayed on the top layer of the column. When the amount of sprayed alga increased, algae flowed down to lower layers, as expected (Fig. 20).

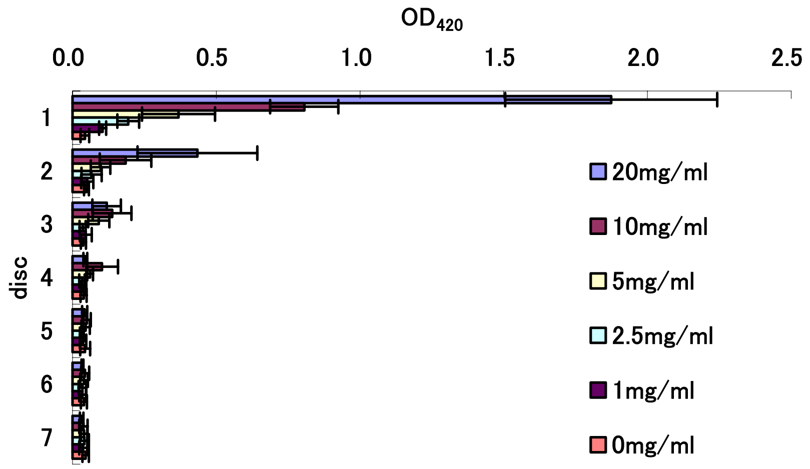


Fig. 20. Distribution of alga sprayed at different concentrations (same volume)

At 3 different amounts of rainfall, after 20mg/ml algae spray on KAK soil column, the chlorophyll of the alga was still found mostly in the top soil disc (Fig. 21). Movement of alga downward rarely occurred. There was no obvious difference which may have caused by the amount of rainfall in the top soil disc.

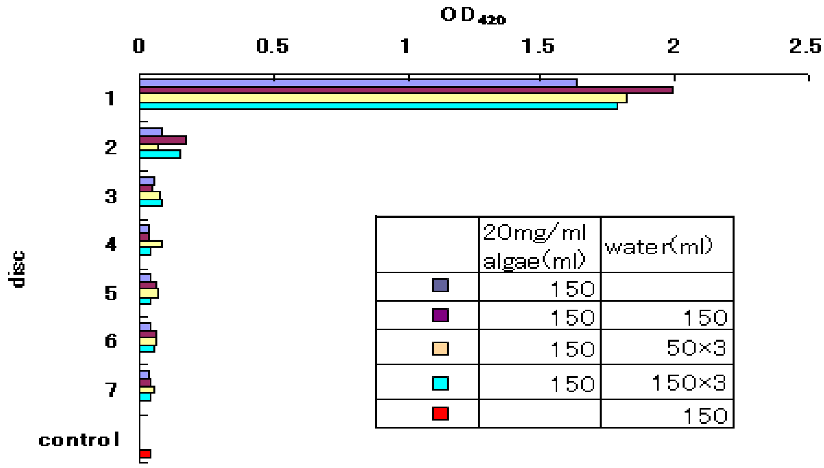


Fig. 21. Location of algae with different amount of rainfall

Ⅳ - 2 Box experiment

Ⅳ - 2 - 1 Physical effects

Ⅳ - 2 - 1 - 1 Effect of algae on reducing soil loss from the surface

0, 5, 10, 15, 20, 30 and 40g algae suspension 150ml (equal to algae amount of 0, 20, 40, 60, 80, 120, 160mg/cm^2) was sprayed on Ta, TK and Ka soil boxes respectively. 3 times of simulated rainfall were sprayed later and each interval was one week.

In Ta, TK and Ka soil boxes, soil loss from those treated with 0 and 5g/box alga were higher than those from treatments of 15-40 g/box (Fig. 22A, 22B and 22C). Soil loss during first rainfall from the box treated with 10 g/box was low, but increased to similar level as the treatment of 5 g/box in the later rainfalls. There was no obvious difference in soil loss in treatments of 15-40g /box. The soil loss decreased greatly and remained low even after 3 rainfalls compare to the control. Soil loss after 3 simulated rainfalls in treatment of 15 g/box algae suspension were reduced by 17%, 12% and 1% of the control in Ta soil, TK soil and Ka soil respectively. The total soil loss (Fig. 23) after 3 times simulated rainfalls in treatments of 15 – 40 g / box (60 - 160 mg/cm^2) algae were 17% - 29% of the control in Ta soil, 12% - 22% in TK soil and 1% - 8% in Ka soil respectively. It seems that sandy loam soil lost less silt than the clay soil did after algae applications.

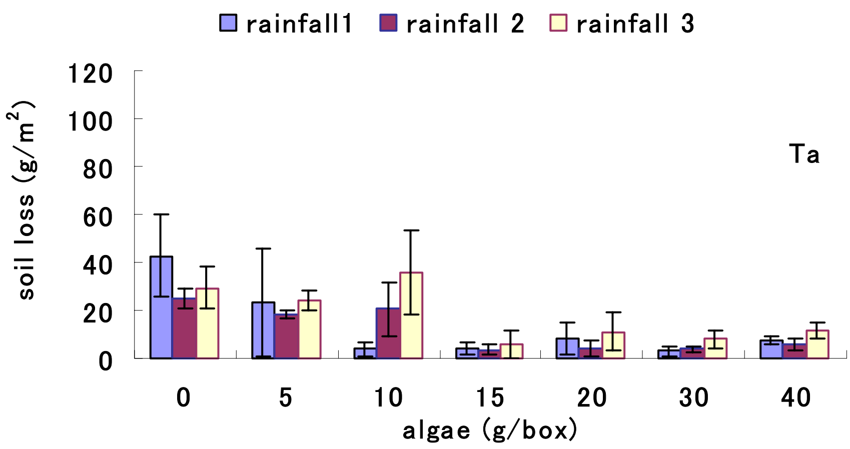


Fig. 22A

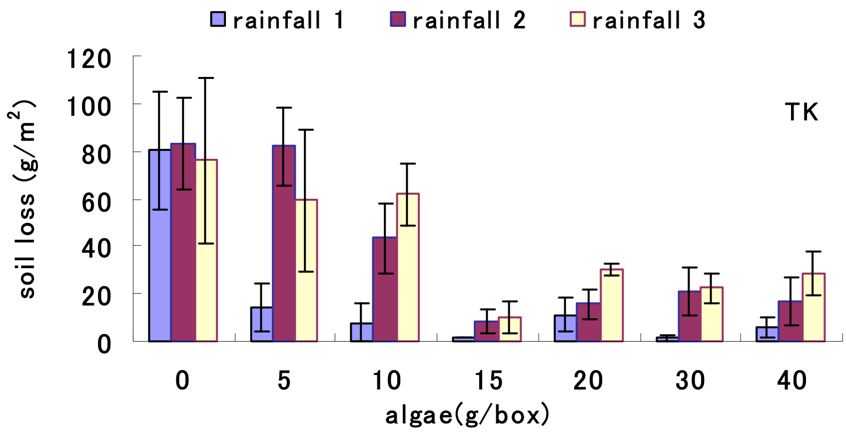


Fig. 22B

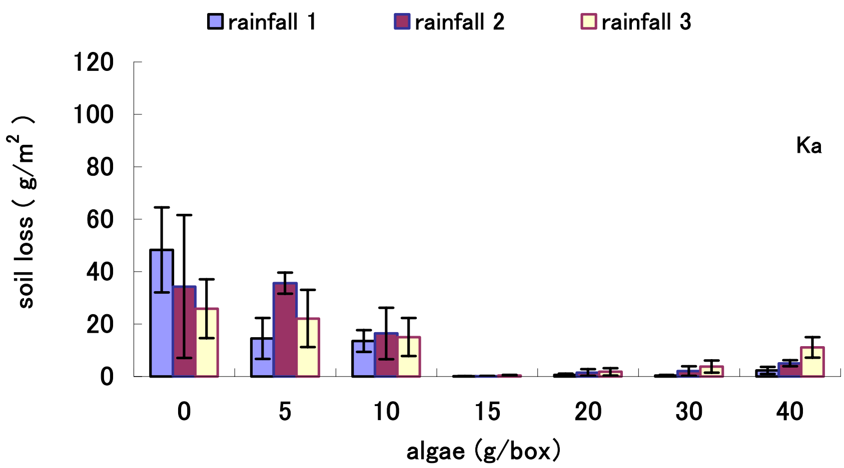


Fig. 22C

Fig. 22 Soil loss from three 20mm watering (simulated rainfall) following algae (0, 5, 10, 15, 20, 30 and 40g) suspension applications (150ml) on Ta soil (A), TK soil(B) and Ka soil (C). Results from Test 1, 2 and 3 were combined. Bars represent standard errors of the mean.

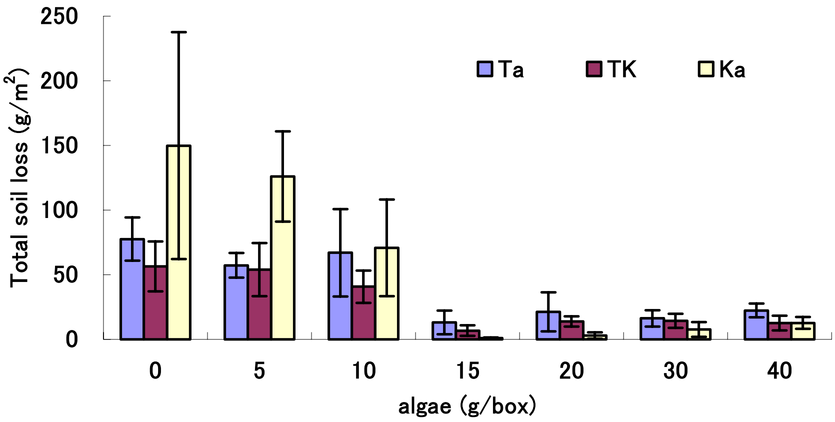


Fig. 23. Total soil loss from three rainfalls following algae (0, 5, 10, 15, 20, 30 and 40g) suspension applications (150ml) on Ta, TK and Ka soil. Bars represent standard errors of the mean.

Ⅳ - 2 - 1 - 2 Effect of algae on the volume of surface runoff

In 3 kinds of soil (Fig. 24A, 24B, 24C) the runoffs after 3 simulated rainfalls at various level of the alga application did not vary significantly even runoff fluctuated in some treatments. Therefore, runoffs in 3 kinds of soil in treatments from 5-40g/box remained stable, indicating that the alga application does not affect the surface runoff during rainfall.

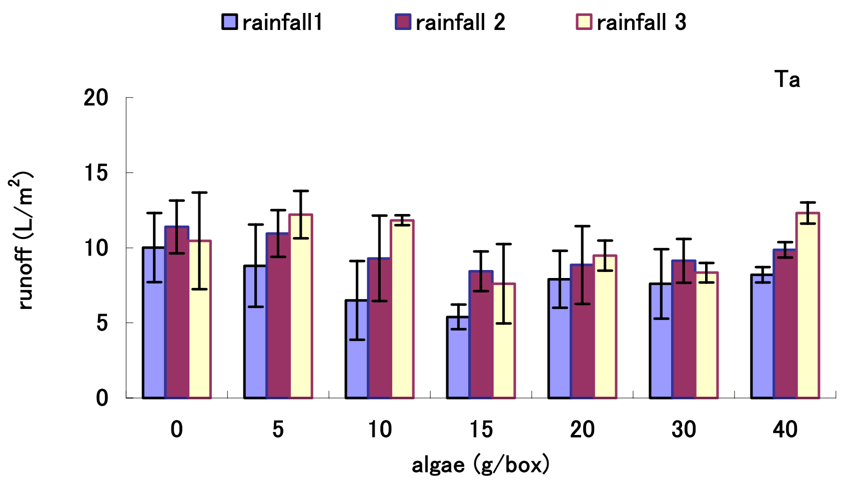


Fig. 24A

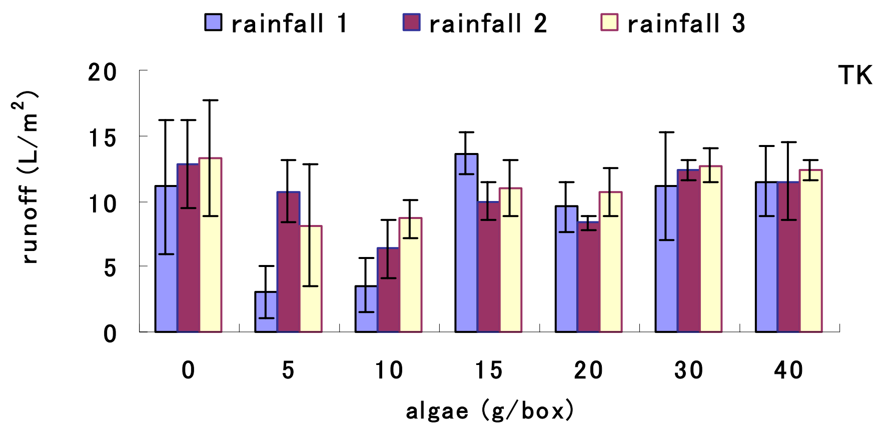


Fig. 24B

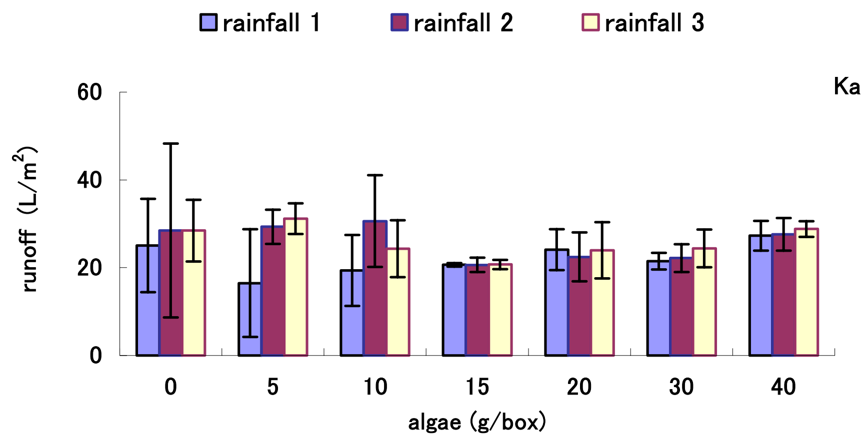


Fig. 24C

Fig. 24. Volume of runoff from three times of simulated rainfall following algae (0, 5, 10, 15, 20, 30 and 40g) suspension applications (150ml) on Ta soil (A), TK soil (B) and Ka soil(C). Bars represent standard errors of the mean.

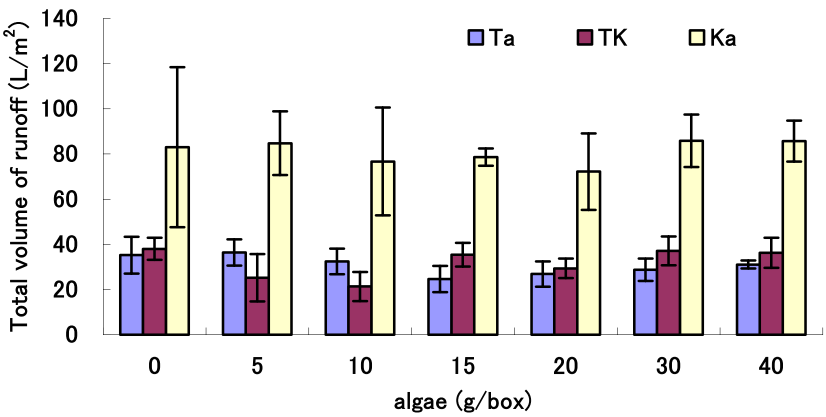


Fig. 25. Total volume of runoff from three times of rainfalls following algae (0, 5, 10, 15, 20, 30 and 40g) suspension applications (150ml) on Ta, TK and Ka soil. Bars represent standard errors of the mean.

Ⅳ - 2 - 2 Chemical and Biological effects

Ⅳ - 2 - 2 - 1 Total organic carbon (TOC) and total saccharide (TSC)

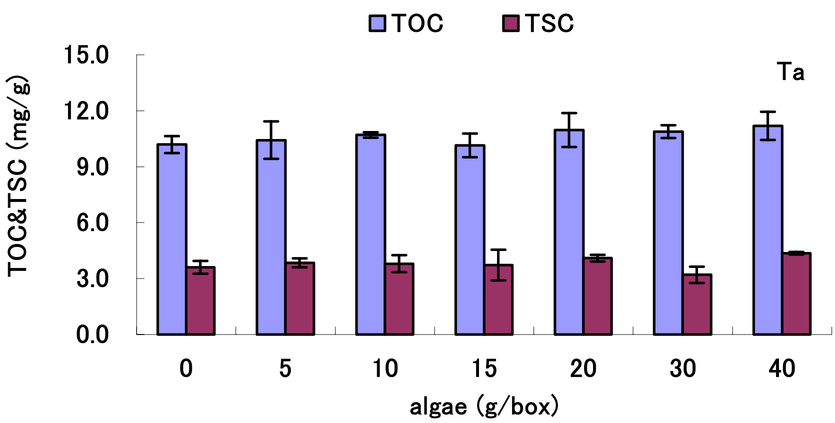


Fig. 26A.

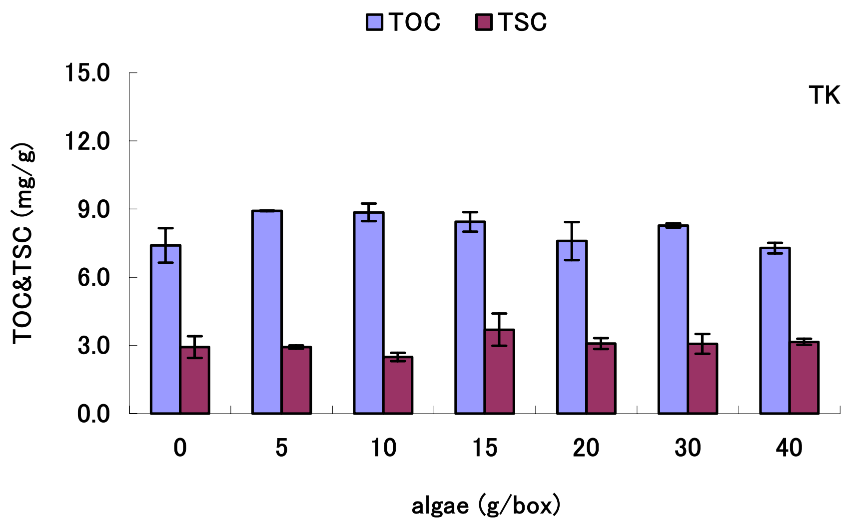


Fig. 26B.

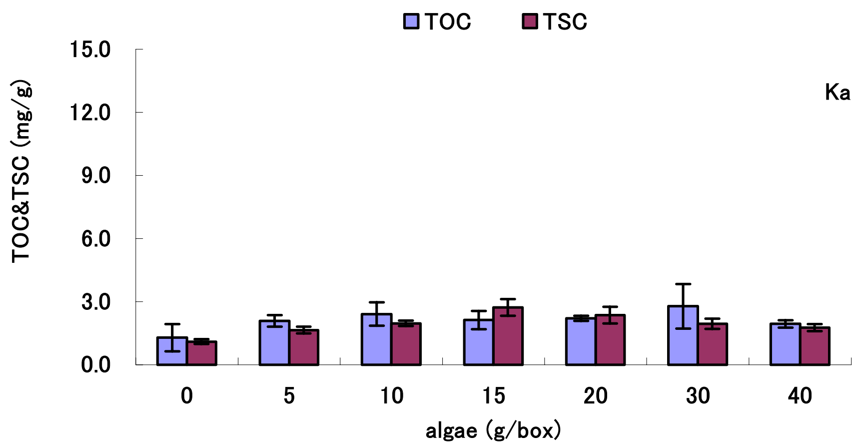


Fig. 26C

Fig. 26. TOC and TSC of the Ta soil (A), TK soil (B) and Ka soil (C) 4 weeks after algae spray concentration of alga was 0, 5, 10, 15, 20, 30 and 40g in 150ml, respectively. Results from Test 1, 2 and 3 are combined. Bars represent standard errors of the mean.

In Ta and TK soil (Fig. 26A, 26B), both TOC and TSC show no significant difference among algae suspension treatments. TOC and TSC in Ka soil (Fig. 26C) increased in all the treatments with algae spray, even at lowest amount. The increase would be meaningful with low original TOC and TSC in Ka soil.

Ⅳ - 2 - 2 - 2 ATP

ATP was determined 4 weeks after algae suspension sprayed. There were 3 times of rainfall after algae spray and the interval was one week. Soil samples were taken from the 1cm deep from the surface.

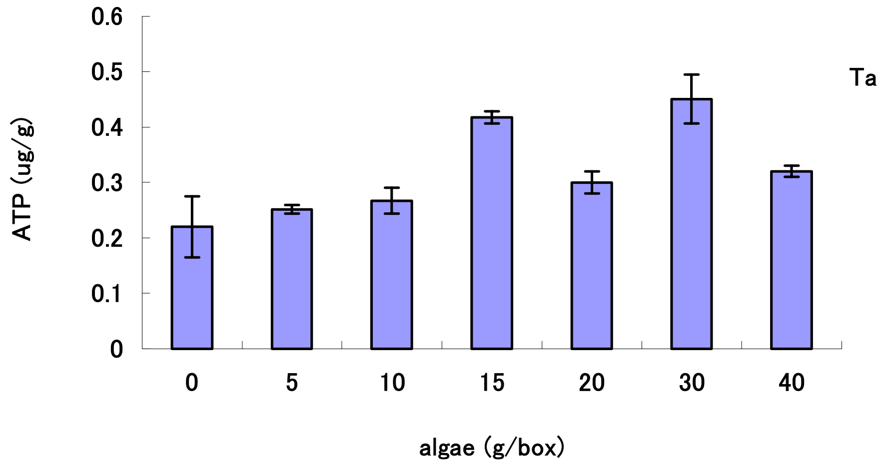


Fig. 27A

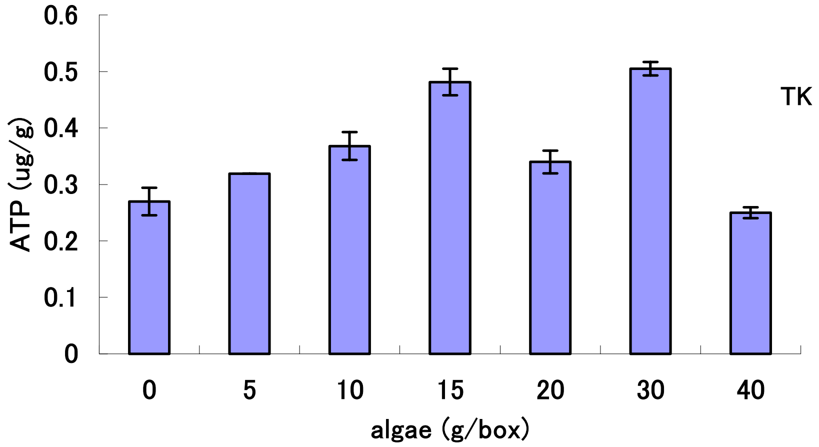


Fig. 27B

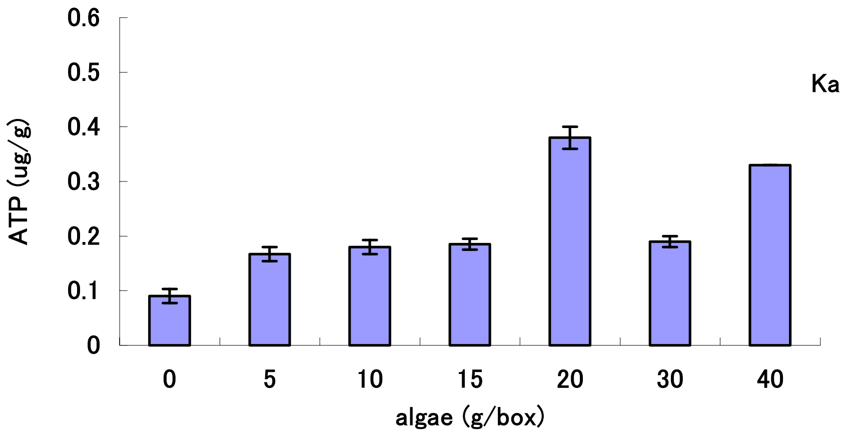


Fig. 27C

Fig. 27 ATP of the Ta soil (A), TK soil (B) and Ka soil (C) 4 weeks after algae spray concentration of alga was 0, 5, 10, 15, 20, 30 and 40g in 150ml, respectively. Results from Test 1, 2 and 3 are combined. Bars represent standard error of the mean.

ATP in 3 kinds of soil treated with algae in different amounts increased significantly (Fig. 27A, 27B, 27C); the maximum of increased ATP were 2 times of the control in Ta and TK soil, and 4 times of the control in Ka soil. ATP reached the largest value in the 20-30g (40-60mg / cm^2) algae spray. Average amount of ATP in all alga treated soil was increased more than 40% of that in the control. It seems that the effect of algae spray on ATP in the soil is larger in less nutrient soil.

Ⅳ- 2 - 2 - 3 Respiration

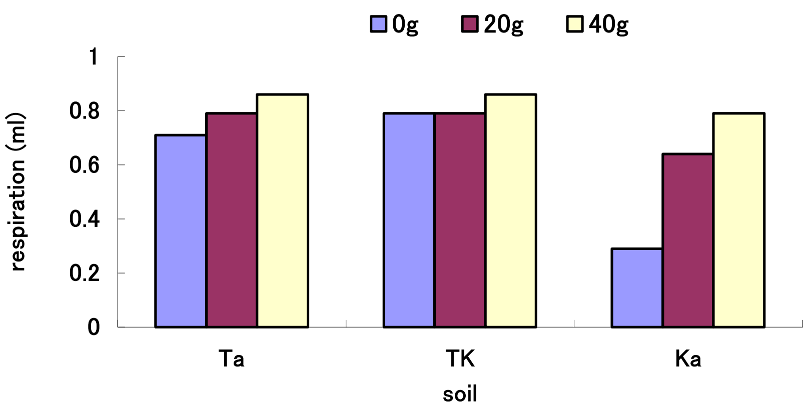


Fig. 28. Soil respiration of 3 kinds of soil 4 week after algae spray concentration of algae was 0, 20, and 40g suspended in 150ml per box

In Ta and TK soil shown in Fig. 28, soil respiration did not vary significantly among different amounts of the algae spray. Soil respiration in Ka soil, on the other hand, increased significantly with increase of the amount of sprayed algae.

V Discussion

In preliminary experiment, the turbidity of outflow from the bottom of the pots filled with soil was greatly less when algae suspension sprayed on the soil surface than that from the water sprayed. Based on the result, our research started to study the possible effect of algae on the reduction of soil loss and on chemical and biological properties of soil.

Polyacrylamide and some synthetic polymers had been applied to reduce soil loss. Authors suggested that the adsorption of the polymers occurred mainly on the external surface of the clay packages. The interaction of the nonionic polymers with the surface is mainly through hydrogen bonds and through various dipole-dipole or charge-dipole interactions. Cationic polymers are adsorbed mainly due to the interactions between the cationic groups of the polymer and the negatively charged clay surface (Theng 1982). Anionic polymers may adsorb on the broken edges of clay by attraction between the negative groups of the polymer and the positive aluminum ions exposed at the broken edges (Greenland 1972). Spray of huge amount of synthetic polymers is not acceptable at present days because of environmental tasks and dying out resources.

The mechanism of water erosion is related to many factors, for example water discharge, average flow velocity, slope, shear stress, bed roughness, particle size and so on. Some of these factors are interrelated and dependent on each other.

Soil particle size is an important factor. Soil particles are graded within a range of conventionally determined sizes, as shown in Table 4. The size distribution of the particles is determined by a method known as mechanical analysis of soil (Garrett, S. D., 1981).

Table 4. Grading of soil mineral particles

Grade	Particle diameter(mm)
Gravel	>2
Coarse sand	2-0.2
Fine sand	0.2-0.02
Silt	0.02-0.002
Clay	<0.002

Particle diameter is one main factor of affecting the soil erosion. Oades and Waters (1991) introduced the concept of aggregate hierarchy. Large aggregates (>2000μm) were hypothesized to be held together by a fine network of roots and hyphae in soils with high soil organic carbon content (>2%), while 20-250μm aggregates consist of 2-20μm particles, bonded together by various organic and inorganic cements. Water stable aggregates of 2-20μm size consist of <2μm particles, which are an association of living and dead bacterial cells and clay particles. It was suggested that organic matter controls aggregate stability and degradation of large (relatively unstable) aggregates creates smaller, more stable aggregates. Koh and Sato (1988) studied the mechanism of rill erosion and suggested soil properties such as cohesion and aggregate stability as important factors. Vehaegen (1984) reported negative correlation between aggregate stability and splash and wash loss.

Tisdall and Oades (1982) proposed that the 'fresh' or 'active' part of soil organic matter (consisting of mono- and polysaccharides, exudates from roots and fungal hyphae) was largely responsible for stabilization of aggregates. However, they found that due to the variability of organic matter, the strength and time for formation of aggregates varied. For example, glucose-like components acted strongly in aggregate formation for the first 2-3 weeks of the experiment, cellulose showed the maximum effect after 6-9 months but was never as effective as glucose. Ryegrass residues were most effective after 3 months and maintained the effect for another 4-6 months, after which the effect declined (Oades et al., 1989). Based on their data, it is apparent that a specific group or groups of organic matter are key agents for aggregate formation and maintenance of structural stability in soils. However, there is no general agreement as to the type of organic matter essential for aggregation. This is most likely due to the fact that different types of organic matter perform different functions at different times during the aggregate formation and conservation process.

The alga, *Phaeocystis* sp. has extracellular polysaccharide which is a nonionic polysaccharide. The mechanism of *Phaeocystis* sp. on reducing silt from surface flow was assumed that glutinous algae stuck to soil silt surface become a binding agent for fine particles to form alga-silt complex, which construct filter layer to decrease vertical infiltration and increase water flow on the surface. From results of 3 kinds of soil, algae benefit on reducing surface silt loss both on clay and sand soil, especially in sand loam soil (Ka soil).

Ⅴ - 1 The results by the column experiment

Ⅴ - 1 - 1 Effect of algae on soil loss from the bottom of the soil column

Degree of soil compactness affects reduction of soil loss with or without algae from the bottom of column (Fig. 16). Results show that soil loss decreased with the increase of packed times at 10mg/ml (0.1g/cm^2) algae spray. Soil loss from the tightly packed soil was much less than that from soft packed soil of loose texture in KAK soil. Compactness of soil seemed no further effect on reducing soil loss at more than 20 times packing. Packing soil column for 20 times was chosen in later column experiments.

Results in Fig. 17 showed that algae spray had good effect on preventing silt flowed out from the bottom of the column. In this study, the effects of reducing soil loss increased with the increase of algae concentration. It suggested that all algae concentrations applied had the ability of reducing flown out silt from the bottom of the column of KAK soil, and that 0.1g /cm^2 was enough to reduce soil loss to a half of the control.

Soil loss from the bottom of the column is thought to be a trigger of gully erosion. Estimation of subsurface flow may be important in surveying where gully erosion will begin and develop. This type of erosion usually occurs in soil containing more sand and less clay soil texture. As there is more gaps among soil particles than in clay type soil, soil shows looser texture, so that more water infiltrates into deeper and transports soil into watercourse. As erosion rills grow larger to gullies, processes such as gravitational collapse (i.e. in Qing Hai) of channel walls and heads will take place. Runoff and sediment from rills and gullies may be moved into ditches, streams and rivers, and soil transported well away from the point of origin.

In Fig. 18, rainfall experiments further proved that algae spray at 0.2g/cm^2 reduced soil loss from the bottom of 10cm deep KAK soil column (2) and that the effect was still obvious at 3 times weak simulated rainfall (50ml × 3 times). No algae effect was found after 3 times strong simulated rainfall (150 ml ×3 times) suggesting that heavy rain will wash out the (effect of) algae. Algae application in reducing soil loss from subsurface can only keep a short term under the condition of strong simulated rainfall. It seems that under weak rainfall the alga sprayed on soil has more chance to build up soil – algae - soil complex to resist to soil loss. It is necessary to conduct more study to prove it.

Reduction of soil loss from vertical infiltration by algae is likely to be that each lower soil layer might be a filter for the flowing suspension and blocks most silts carried by upper water body. When algae suspension is applied on the soil, algae may infiltrate along soil gaps, to reduce the size of soil gaps, adhere to soil particle surface, finally to form a gelatinous filter layer and block silt flowing down to the subsurface.

Vertical infiltration is likely to be limited by the gaps/pores of soil. If rain continues, infiltrating flows enlarge pores /gaps in soil and give energy (velocity) to water flow and so more silt is carried away. When algae sprayed on the soil surface, more algae will flow in the soil, so that, the infiltrating speed decrease, and the water on /near the surface gradually increased and accumulated. The water flow becomes slower. The reduced water flow with less energy can not carry more silt to flow out.

Calculation resulted that the algae (3g, wet) applied in a column were enough to construct an alga layer on the surface to decrease the velocity of rainfall drops on the soil surface when it was supposed that algae cells did not infiltrate. Depending on the relationship of the number of algae cells and their dry weight: Cells = $4.74 \times DW_{algae}$ (Liu Yingchun, to be published), the number of cells in 2% algae suspension of 150ml was 3.7×10^{9}, and might cover on the surface about 1.9mm thick in the column(the diameter of alga is average 5μm). The effect of reducing soil loss from the bottom of the column may be understood that the algae layer decreases energy of raindrop splash and reduces the velocity of rain infiltration.

Ⅴ - 1 - 2 Distribution of algae in soil column

The results of distribution of algae in soil columns also showed that most of algae at all algae concentrations were held on the disc of the top soil in KAK soil (Fig. 19a). Fig. 19b suggests that larger silt particles may be formed with the sticky alga, and deposited easier than the fine silt particles during centrifugation which gives turbidity. Fig. 20 showed only small part of high concentration algae can infiltrate to the 2-3cm deep. Even simulated heavy rainfall can not improve algae infiltrating deeper (Fig. 21). Direct observation also found in algae spray at high concentrations that most algae were blocked on the surface of soil column to construct a gel-like layer which resulted in algae suspension slowly permeate down into soil. The alga, *Phaeocystis* sp. has extracellular polysaccharide which is a nonionic polysaccharide and may work as a binding reagent for fine particles of soil to make clots then to block the gaps/ pores in soil. Water of algae suspension and rainfall water later slowly permeated the gel-like layer and flowed out with less silt.

Ⅴ - 1 - 3 Effect of algae on reducing soil loss from the surface

Results in Fig. 22A, Fig. 22B and Fig. 22C show all concentrations of algae spray of 5-40g / box (20, 40, 60, 80, 120 and 160mg/cm^2) had benefit effect of reducing soil loss more than half of the control in the first simulated rainfall in three soils. Soil loss from the surface was greatly reduced after three times of simulated rainfall following spray of algae suspension especially at concentrations higher than 15g / box on 3 kinds of soil. Soil loss during the 3rd simulated rainfall after 60 - 160 mg/cm^2 algae sprayed were less than 50% of the control in all three soils. The total soil loss after 3 times simulated rainfalls in treatments of 60 - 160 mg/cm^2 algae were 17% - 29% of the control in Ta soil, 12% - 22% in TK soil and 1% - 8% in Ka soil respectively (Fig. 23). Algae spray seemed to give better effect in reducing soil loss on sandy loam soil (Ka soil) than on the clay soil (Ta and TK soil).

In all three soils, 15g/box (60mg/cm^2) showed the best effect of reducing silt loss. Calculation using the method described in Ⅴ-1-1 resulted that 15g wet algae included 1.45×10^{12} algae cells can cover the soil surface (256cm^2) by 0.57mm thickness if algae infiltration was not considered. Algae spray at 5g/box and 10g/box will give a surface algae layer of 0.19mm and 0.38mm thick, respectively. An algae layer for 0.57mm may be just enough to be resistant to the splash rain drops for 3 times.

The volume of surface runoff in all three soils was almost not affected with algae spray (Fig. 24A, Fig. 24B and Fig. 24C). This can be explained that the sprayed algae formed algae gelatinous cover on soil surface then limited the rain water infiltrating into the soil and so resulted in flowing out along the surface. Only with algae spray at lower concentrations algae spray seemed to reduce some extent of runoff during the first rainfall. They may not form a layer which blocks infiltration and increase the volume of surface runoff. However, the total volume of runoff from three rainfalls also shows no difference between the algae spray and the control (Fig. 25).

Ⅴ - 1 - 4 The effect of algae applied on chemical and biological properties in 3 soils

Total carbon in soils is the sum of organic and inorganic carbon. Most organic carbon is present in the soil organic matter, while inorganic carbon is almost in carbonate mineral. Organic carbon is contained in the soil organic fraction, which consists of the cells of microorganisms, plant and animal residues at various stages of decomposition, stable 'humus' synthesized from residues, and highly carbonized compounds such as charcoal, graphite, and coal (elemental forms of carbon) (Page, A. L. et al, 1982). The erodibility of most soils is closely linked to their organic content. Conventionally, total organic carbon (TOC) is assumed to 58% of total organic matter (Landon, 1984).

Soil organic matter and specifically soil total organic carbon are known to play important roles in the maintenance as well as improvement of many soil properties. The biological functions of soil organic materials are primarily to provide a reservoir of metabolic energy that drives biological processes, to act as a supply of macro-and micro-nutrients and to ensure that both energy and nutrients are stored and released in a sustainable manner. Soil organic materials are usually expressed by TOC.

Total saccharide refers a series of compounds composed of carbon, hydrogen, and oxygen especially those containing the group CHO. Saccharide is one of excellent source of energy for soil organisms, similar as protein and nucleic acids. It can be rapidly decomposed when added to soil unless they enter into complex formation with soil colloids.

Results show that both TOC and TSC had no significant difference among different algae treatments in Ta soil and TK soil (Fig. 26A and Fig. 26B). The reason might be the amount of additions was not enough to affect the original status. However TOC and TSC in Ka soil (Fig. 26C), the levels of which are quite low, increased in all the treatments with the alga suspension, even with lower concentration. It suggests that algae spray increases both soil TOC and TSC in infertile soil.

Adenosine 5'-triphosphate (ATP) has been widely used as an indicator of living organisms. ATP occurs in all living cells and can be measured by the luciferin-luciferase system. The ATP-dependent oxidation of luciferin by luciferase produces light which can be measured by Lumicounter. When ATP is the limiting factor in the luciferin oxidation reaction, the amount of light produced is proportional to the amount of ATP of the sample. ATP has been used as a measure of soil microbial biomass or microbial activity in different ecosystems as well as in the sediments and soils (Lee et al., 1971; Oades and Jenkinson, 1979; Sparling, 1981; Fairbanks et al., 1984; Arnebrant and Schnürer, 1990; Lin and Brookes, 1996).

The results shown in Fig. 27A, Fig. 27B and Fig. 27C suggest that ATP in 3 kinds of soil after different concentrations of algae spray increased significantly. Average amount of ATP in all of the algae treated soil increased more than 140% of the control. The amount of the soil treated with the alga at 5 – 40 g/box (20 – 160 mg/cm^2) increased to 116% - 204% of the control in Ta soil, 100 - 202% in TK soil, and 186% - 428% in Ka soil. It was clear that ATP in barren soil as Ka soil increases much more than in other 2 types of soils and reaches to the similar level. Increase of ATP suggests the action of soil microbes, which is very important for improvement of soil nutrition. Various microbes are the most important component in soil, which affects the decomposition of organic matters to be humus. Humus supply nutrient to the growth of plants, and combine minerals to form soil aggregates to improve soil structure. The addition of organic matter in algae and action of microbes are crucial means of improving soil fertility. The algae spray showed the results of both TOC and ATP increase in barren soil.

Soil respiration means the uptake of O2 and / or the release of CO2 by metabolizing organisms in the soil. Soil respiration closely links to the ATP content of the soil. Both parameters are expression of the activities of soil microbes.

In this study, the real–time soil respiration was measured with newly invented electronic micro–manometer. The results shown in Fig. 28 indicated that soil respiration especially in Ka soil increased with increase of the amount of sprayed algae. The results suggest that the soil respiration after the algae spray increases much more in less nutrient soil than in the soil with better nutrient status. These results corresponded well to the results of soil ATP.

Ⅴ - 1 - 5 General effects of algae spray in the box experiment

The biological functions of soil organic materials are thought primarily to provide a reservoir of metabolic energy that drives biological processes, to act as a supply of macro-and micro-nutrients and to ensure that both energy and nutrients are stored and released in a sustainable manner. Importantly, biological processes in turn influence both soil chemical and structural properties of soil as they greatly affect soil structure and soil redox reactions.

Soil box experiments were used to simulate soil loss from surface following simulated rainfall and to estimate effects of algae on soil chemical and biological properties 4 weeks after the algae was sprayed. Similar results obtained showed that algae suspension application reduced soil loss from the surface in 3 soils, increased soil

TOC and TSC significantly in Ka soil and increased ATP in 3 soils, especially greatly in Ka soil.

Fig. 29A reorganized the results of the amounts of silt in surface runoff, ATP and soil respiration in Ta soil. The amount of silt in surface runoff showed significantly decreased with algae spray (20, 40g). The algae spray at 20 and 40g/box can reduce 3/4 of soil loss of the control. However soil ATP and respiration of soil microbes showed relatively small increase.

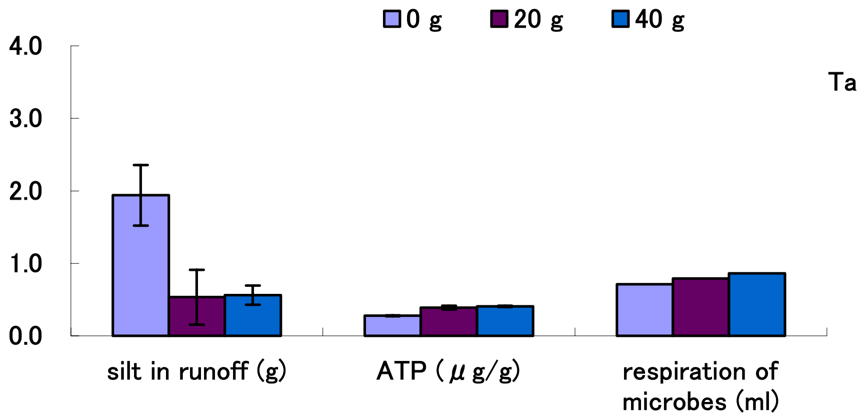


Fig. 29A.

In TK (Fig. 29B), the amount of silt in surface runoff also showed significantly decreased with algae spray (20, 40g). Other features including ATP and soil respiration resembled with the results of Ta soil (Fig. 29A).

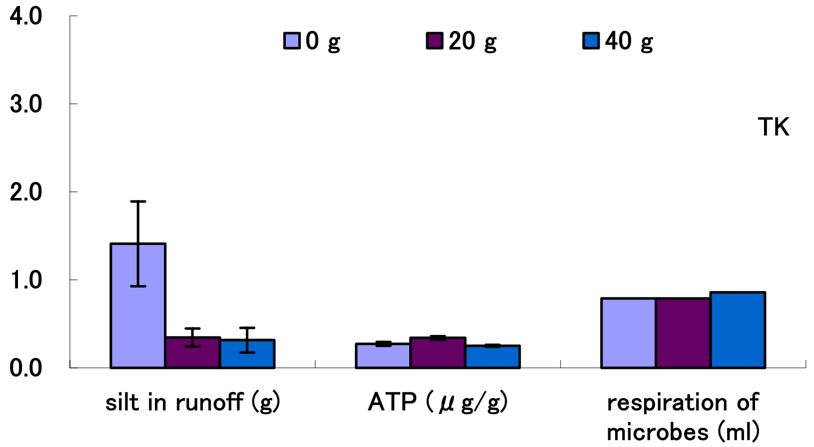


Fig. 29B

In great contrast to the results of the Ta and TK soil, it is clear that in Ka soil (Fig. 29C), the amount of silt in surface runoff showed greatly decreased. This is due to the amount of soil in surface runoff in Ka soil is much larger than that of TK and Ta soil Even so, algae spray depressed the amount of soil loss to the level obtained with Ta and TK soil. In contrast, Soil ATP and the respiration of soil microbes showed considerable increase by algae spray with an increase of the amount of algae.

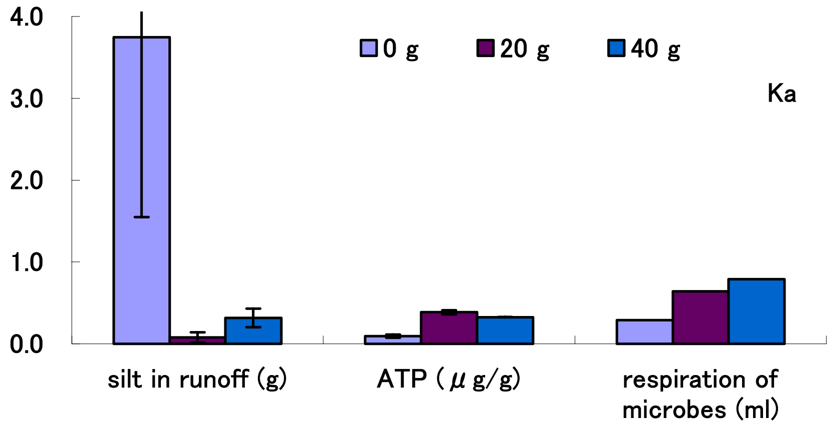


Fig. 29C

Fig. 29 Variation of soil silt in surface runoff, ATP content and soil microbes respiration amount 4 weeks after 0, 20 and 40g algae suspension (150ml) spray on Ta soil (A), TK soil (B) and Ka soil (C) and in this period 3 times of simulated rainfall in one week interval were applied. Bars represent standard errors of the mean.

Soil structural stability refers to the resistance of soil to structural rearrangement of pores and particles when exposed to different stresses. The interrelationship between soil organic carbon and soil structure and other physical properties has been extensively studied. It is well established that addition of soil organic materials can reduce bulk density, increase water holding capacity and effectively increase soil aggregate stability.

Properties of 3 kinds of soil in this box experiment are listed in Table. 5. TOC of 3 soil samples are less than 2%, especially Ka soil holds the extremely low organic carbon. In other words, there is almost no nutrient in Ka soil. Algae spray improved microbial biomass in barren Ka soil as suggested by increase in both soil ATP content and soil respiration.

Ⅴ - 1 - 6 Soil structure and the effects of algae spray

Properties of five soils are listed in Table 5. Textures are shown in Fig. 30 by the USDA textural triangle.

Table.5 soil properties of 5 soils

soil	Ta	TK	Ka	KAK	Qinghai
pH	5.5	5.4	5.2	5.6	7.6
texture(clay/silt/sand) (fine sand / course sand)	54/32/14	40/31/29	5/26/69 (28+41)	12/26/62 (17+45)	14/23/63 (58.3+4.2)
TOC (mg/g)	11.3	6.2	1.3	13.7	0.9
TSC (mg/g)	4.6	2.2	0.7	ND	ND
soil density (g/ml)	1.6	1.3	0.9	ND	ND
weight (kg/box)	5.4	4.3	2.8	ND	ND
soil type	clay	clay	sandy loam	sandy loam	sandy loam

ND: not detectable

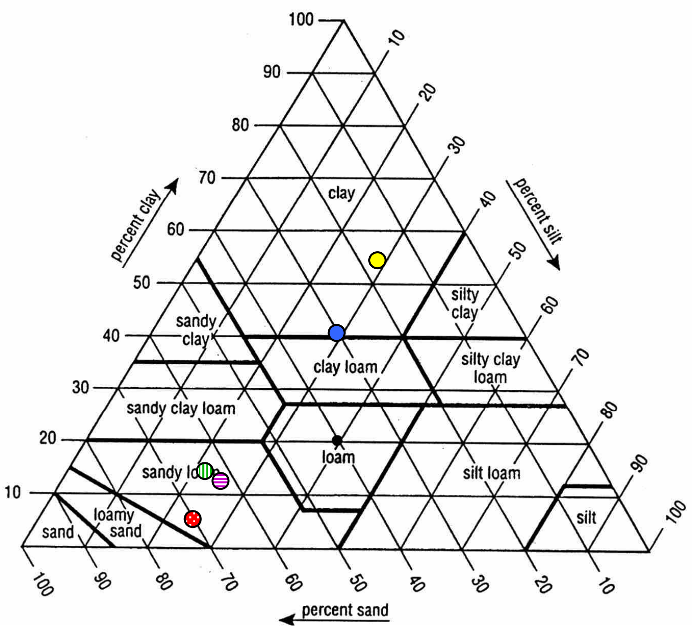


Fig.30. Compositions of five soil are described in the USDA textural triangle

○ Ta soil; ● TK soil; ● Ka soil

● Qinghai soil; ● KAK soil

TK, mixture of Ta and Ka (1:1) should show the average of Ta and Ka soil. The difference shown in Fig. 30 and Table 5 came from the adjusting of soil moisture. So in the mixture there was more clay than the theoretic proportion. Between Ta soil and Ka soil, the textures had a significant difference. Ka soil was almost 70% sand and Ta soil was only 14%. One of main factors of affecting soil loss was clay content which in Ta soil was 54% and in Ka soil was only 5%. *Phaeocystis* sp. showed beneficial effect of reducing soil surface loss with simulated rainfall in 3 kinds of soil in the study. Soil of Ping An in Qinghai is similar to Ka and KAK soil in structure. They are sandy loam; the soil loss in Qinghai is likely to be vertical soil loss. The results of the column experiment with KAK soil indicated that algae might have effect of reducing soil loss in Qinghai soil and increasing soil activity of microbe.

Ⅴ - 2 Concluding remarks

Spraying of *Phaeocystis* sp. suspension could have better effect in sandy loam soil than in clay rich soil. The reason for the beneficial effect of polysaccharide was thought to be aggregate stability due to the formation of silt-algae clot. The algae, *Phaeocystis* sp. adhered to silt surface to construct macro aggregate, decreased pore size and/or stuck together covering on silt to decrease the surface exposed to the raindrops. In the column experiment, algae were shown to stay on the top layer of soil and decrease water infiltration. In the box experiment, even though the amount of algae was not enough to cover the entire surface, the effect possibly came out by forming a temporary silt-algae clot to prevent silt loss from surface flow. Plants residues covered the field can have long term effect of reducing soil loss (Lal, R. 1998, Zheng, F. L. 2004). If plants residues were combined to algae spray, effect of preventing soil loss could be increased greatly and would extend to long period not only a short term. As biological activity / amount in less nutrient soil were increased greatly after algae spray, for example in Ka soil, algae spray not only reduces soil loss, but also increases microbial biomass. This is a prospective method for improving barren land and reducing soil loss.

The soil box experiments were conducted with a box of the surface area about 250cm^2, the length of slope 23cm. In this case, the box wall would produce more influence on water flowing. Sometimes water was flowed down along the wall rapidly if soil can not adhere tightly to the wall. So in my experiment, the soil was packed carefully to avoid the gaps between soil and wall. Even so, the soil density can not be simulated to the natural state. Another factor of soil loss was the length of slope. As known, longer and steeper slope could produce more soil loss. Larger soil box should be used to investigate more on the effect of algae. Other soil texture type soil can also be examined to enlarge the applying range.

As in the USDA textural triangle shown in Fig. 30, the soil of Qinghai in China was also listed. China has catastrophic erosion along Yellow River. The headwaters of Yellow River are in Qinghai although the most serious passage of soil loss is not in Qinghai. Erosion in some areas of Qinghai is also serious. And its soil is close to the Ka soil which was greatly affected by algae spray. Qinghai has many salt lakes with different level of salt concentration. This salt water can be used to culture *Phaeocystis* sp.. After simple process, algae suspension can be applied on the deteriorated soil to reduce soil erosion both on the surface and vertical soil loss. The algae were natural product and can be taken account to be nutrient of soil microorganisms and increased soil organic matter. We can image the effect of algae might have benefit effect in Qinghai soil.

Microorganisms in deficient nutrient soil in Qinghai as well as in Ka soil would greatly increase. We hold a prospect that this method can be used in Ping An of Qinghai for improving poor soil and reducing soil loss.

Acknowledgement

It is now when I am about to leave Kochi University of Technology, where I have spent three years conducting this research. This research would not be possible without direct and indirect assistance from many people.

Sincerely, I would like to express my profound gratitude to my advisor, Prof. Dr. Yasuo Mukohata for his valuable and helpful advice, warm encouragement, and continuing support throughout course of this research work. I would like to thank for his efforts leading me when I was struggling, and for his patience explaining me when I was confused. It is very fortunate for me having chance to be his student and worked at KUT.

With my great honor to have Prof. Nobuya Matsumoto, Prof. Keiichi Enomoto, Associate Prof. Osamu Ariga, and Lecturer Sakae Horisawa serving as my research examination committees, I would like to thank for their useful discussions, constructive comments, and valuable suggestions.

I would like to thank Ms. Hiromi Seki, Ms. Asuka Kobayashi, Mr. Nobukazu Nagamatsu, and Ms. Syouko Okamoto for their experiences, support and helps. Sincere thanks are also extended to Ms. Liu Yingchun for her valuable advice and amendment in writing the dissertation and Ms. Xie Zhanling for her academic and technical helps. Many thanks are also extended to other students, Mr. Masayuki Teshima, Mr. Hiroyuki Sato, Ms. Ayaka Tsujii and Ms. Yusie Kohashi for their helps and supports. I also would like to thank Mr. Hideto Emura, Mr. Toshiyuki Kurachi and Mr. Ryuusuke Kanbe for their kind helps and supports when I started my study and work in KUT. I appreciate all of their friendship and encouragement. All of our cooperative activities and experiences will always remain in my memory.

I would like to thank my Japanese teacher, Ms. Kubo. I also would like to thank the faculties at the International Relation Center for their efforts and cooperation.

I wish to express my gratefulness to Kochi University of Technology, for awarding the special scholarship to pursue the doctoral study.

Last but not least, sincere thanks are also extended to my family for their understanding, encouragement and assistance. Especially to my husband and my daughter, they always concern, support and encourage me throughout my study and stay in Japan. I eternally love them.

References

Arnebrant, K., Schnürer, J., 1990. Chages in ATP content during and after chloroform fumigation. Soil Biology & biochemistry 22, 875-877.

Ben-Hur, M., and R. Keren., 1997. Polymer effects on water infiltration and soil aggregation. Soil Sci. Soc. Am. J. 61:565-570.

Brown, M. J., C. W. Robbins, and L. L. Freeborn., 1998. Combining cottage cheese whey and straw reduces erosion while increasing infiltration in furrow irrigation. J. Soil and Water Conserv. 53(2): 152-156

Fairbanks, B.C., Woods, L.E., Bryant, R.J., Elliot, E.T., Cole, C.V., Coleman, D.C., 1984. Limitations of ATP estimates of microbial biomass. Soil Biology & biochemistry 16, 549-558.

Greenland, D. J., 1972. Interactions between organic polymers and inorganic soil paricles. In Proceedings Symposium on the Fundamentals of Soil Conditioning. M. DeBoodt (ed.). State University of Ghent, Faculty of Agricultrual Sciences,

Kirkby, M.J. and Morgan, R. P. C., 1981. Soil Erosion, John Wiley & Sons, Ltd.

Koh, M. K. and K. Sato., 1988. Studies on the mechanism of rill initiation and development. Trans. JSIDRE Vol. 136:1-7

Kurachi, T., 2003. An attempt to create a plantation pile - Evaluation of materials for the pile and microorganisms for its fertilization. Master dissertation of Kochi University of Technology.

Lal, R., 1998. Mulching effects on runoff, soil erosion, and crop response on alfisols in western Nigeria. J. Sustainable Agric. Vol. 11, no. 2-3, pp. 135-154.

Landon, J. R. (ed.), 1984. Booker Tropical Soil Manual. A Handbook for Soil Soil Survey and Agricultural Land Evaluation in the Tropics and Subtropics, Longman, London.

Lee, C. C., Harris, R. R., Williams, J .D. H., Armstrong, D. E., Syers, J. K., 1971. Adenosine triphosphate in lake sediments: I. Determination. Soil Science Society of America Proceedings 35, 82-86.

Lentz, R.D., and R.E. Sojka. 1994. Field results using polyacrylamide to manage furrow erosion and infiltration. Soil Sci. 158:274–282.

Levin, J., M. Ben-Hur, M. Gal, and G.J. Levy., 1991. Rain energy and soil amendments effects on infiltration and erosion of three different soil types. Aust. J. Soil Res. 29:455-465.

Levy, G. J., M. Agassi., 1995. Polymer molecular weight and degree of drying effects on infiltration and erosion of three different soils. Aust. J. Soil Res. 33:1007-1018

Lin, Q., Brookes, P.C., 1996. Comparison of methods to measure microbial biomass in unamended, regrass-amended and fumigated soils. Soil Biology & biochemistry 28, 933-939.

Morikawa, A., 2001. A use of polysaccharide from unicellular marine alga *Phaeocystis* sp.. Doctor dissertation of Kochi University of Technology.

Oades, J. M., Gillman, G. P., and Uehara, G., 1989. Interactions of soil organic matter and variable-charge clays. In 'Dynamics of soil organic matter in tropical ecosystems. (Eds D. C. Coleman, J. M. Oades, and G. Uehara.) pp. 69-95. (University of Hawaii Press: Honolulu.)

Oades, J.M. and Jenkinson, D.S., 1979. Adenosine triphosphate content of the soil microbial biomass. Soil Biology & Biochemistry 11, 201-204

Oades, J.M. and Waters, A.G., 1991: Aggregate hierarchy in soils. *Australian Journal of Soil Research* 29, 815-828.

Odum, E. P., 1959. Fundamentals of Ecology. W. B. Saunders Co., Philadelphia, Pennsylvania.

Okahana, N., 2002. An attempt to make biodigradable plastics using exocellular polysaccharide of *Phaeocystis* sp. Master dissertation of Kochi University of Technology.

Orts, W.J., Sojka, R.E., Glenn, G.M. and Gross, R.A., 1999. Preventing soil erosion with polymer additives. Polymer News. December, 1999, Vol. 24, pp.406-413

Orts, W.J., R.E. Sojka, and G.M. Glenn., 2000. Biopolymer additives to reduce soil erosion-induced soil loses during irrigation. Industrial Crops &Products. 11:19-29

Orts, W.J., R.E. Sojka, G.M. Glenn, and R.A. Gross., 2001. Biopolymer additives for the reduction of soil erosion losses during irrigation. Pages 102-116. In: R.A. Gross and Carmen Scholz (eds.) Biopolymers from polysaccharides and Agroproteins. ACS Series 786. Am. Chem. Soc., Washington, DC.

Orts. W.J., Soika, R.E., and Glenn, G.M., 2002. Polymer additives in irrigation water to reduce erosion and better manage water infiltration. Agro Food Industry Hi-tech. 13(4):37-41.

Page, A.L., et al, 1982. Methods of soil analysis. Part 2- chemical and microbiological properties. Second edition. The American Society of Agronomy, Inc . Wisconsin, USA

Seki, H., 2004. A simple handy water – purifying powder " MIZUSUMASHI". Master dissertation of Kochi University of Technology.

Shainberg, I. and Levy. G. J., 1994. Organic polymers and soil sealing in cultivated soils. Soil Sci. 158:267-273

Smith, H. J. C., Levy, G.J. and Shainberg, I., 1990. Water-droplet energy and soil amendments: Effect on infiltration and erosion. Soil Sci. Soc. Am. J. 54:1084-1087.

Sparling, G.P., 1981. Microcalorimetry and other methods to assess biomass and activity in soil. Soil Biology & Biochemistry 13, 93-98.

Theng, B. K. G., 1982. clay-polymer interaction: Summary and perspectives. Clays Clay Miner. 30:1-10

Tisdall, J. M. and Oades, J. M., 1982. Organic matter and waterstable aggregates in soils. Journal of Soil Science **33**: 141-163.

Verhaegen, T. H., 1984. The influence of soil properties on the edibility of Belgian loamy soils: A study based on rainfall simulation experiments. Earth surface processes and landforms. Vol. 9:499-507

Vollenweider, R. A., 1968: Scientific fundamentals of the eutrophication of lakes and flowing waters with particular reference to nitrogen and phosphorus as factors in eutrophication. Organization for Economic Cooperation and Development, Directorate of Scientific Affairs, Paris. Report. 220 pp.

Yamashita, S., 2002. On the exocellular polysaccharide of *Phaeocystis* sp.. Master dissertation of Kochi University of Technology.

Zarchar, D., 1982. Soil erosion. Amsterdam ; New York : Elsevier Scientific Pub. Co.

Zheng, F. L., et al, 2004. Runoff, Soil Erosion, and Erodibility of Conservation Reserve Program Land under Crop and Hay Production. Soil Sci. Soc. Am. J. 68:1332-1341