Evaluation of Hyper-Thermophilic Aerobic Compost Produced from Sewage Sludge on Rice Yield

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Abstract

Large amount of sewage sludge generated in the world is necessary to be treated properly and effectively, otherwise, it would be resulted in severely environmental pollution. Hyper-thermophilic aerobic compost (HTAC) is an innovative method for sludge treatment. HTAC was produced by composting sewage sludge inoculated with *C. yamamurae* which was isolated from Kirishima hot spring in Kagoshima prefecture, Japan. However, there is no published paper that reported the effect of HTAC on plant growth when being applied to farmland. Submerged plants taken from lakes also have the potential to be used as organic fertilizers, which could be termed as green manure. The fertilizer potential of submerged plant also has not been studied. Thus, this study aims to elucidate the applicability of HTAC and its optimum fertilization amount for rice growth and yield during 2 years' experiments. The fertilizer potential of submerged plants *Ceratophyllum (C.) demersum* and *Egeria densa* with the same nitrogen content at different ratios were also analyzed using rice growth experiment.

In order to investigate the stability and maturity of HTAC, seed germination test and Komatsuna cultivation in a soilless culture were conducted using Komatsuna seeds. Germination index (GI) value during germination test, and the characteristics of roots and leaves of the cultivated Komatsuna were measured to indicate the safety and quality of HTAC. The rice growth experiments were performed in 2016 and 2017 on a farmland at Tsukuba, Ibaraki, Japan. The optimum fertilization amount of HTAC was clarified, and the fertilizer potential of submerged plants *C. demersum* and *Egeria densa* also were investigated and compared with HTAC. The results obtained can be summarized as follows:

(1) The results from seed germination test indicate that HTAC has no phytotoxicity and can improve the elongation of plant stem. The GI value was higher than 80% when the filtrate of HTAC diluted by 20 times (electric conductivity =2.9 mS cm^{-1}).

(2) HTAC was successfully used in soilless culture system for Komatsuna cultivation. The addition of HTAC at 5.32 g/500 mL not only increased the yield of Komatsuna, but also increased the weight of root.

(3) During the rice growth experiment by applying HTAC, the stem height, tillers number and rice yields significantly increased with the increase in nitrogen application levels. The optimal fertilization amount of HTAC was deemed as 180 kg N ha^{-1} when considering the plant quality and environmental friendliness. The rice growth experiment in 2017 showed the similar trend compared with that in 2016.

(4) The stem height, tillers number and rice yields in the test groups fertilized with the mixture of HTAC and submerged plants significantly increased compared with the control (none fertilizer). While, the number of panicles in R7(180 kg N ha^{-1} of *Egeria densa*), R8 (120 kg N ha^{-1} of *Egeria densa*, 60 kg N ha^{-1} of HTAC) and R9 (60 kg N ha^{-1} of *Egeria densa*, 120 kg N ha^{-1} of HTAC) was 14.75, 13.00, 14.50, respectively. In R8, especially low value was observed, and during the same period the plant height in R8 was also lower than R7 and R9. Thus, when applying *Egeria densa* with HTAC as organic fertilizer, it is necessary to optimize the mixing ratio with HTAC. Submerged plants *C. demersum* and *Egeria densa* were also found to enhance the rice yield significantly, which can be used as organic fertilizer. These two plants have the same effects as HTAC at the same nitrogen level.

Therefore, the results obtained from this study provide significantly important reference for nutrients utilization in sewage sludge treated by HTAC.

Keywords: Hyper-thermophilic aerobic compost; *Ceratophyllum demersum*; *Egeria densa*; Rice growth; Yield components.

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Chapter 1 Introduction

1.1 Overview

Waste activated sludge generated from wastewater treatment plants (WWTPs) may be considered to be a problem. Due to the presence of diverse pollutants, it would become a source for secondary environmental pollution (Raheem et al., 2018). Therefore, its proper disposal and treatment carries utmost significance. From Fig 1-1, in Japan, nearly 60% of sewage sludge were disposed by landfill, or used as blocks and cement, only more than 10% was used as fertilizer and fuel. Meanwhile, anaerobic digestion (AD) and compost are the common technologies for sludge treatment (Tyagi and Lo, 2013).

Landfill may be associated with long-term risks for the environment and public health, as sludge contains heavy metals, pathogens and organic pollutants that have potential to be transferred to plants, livestock and humans (Spinosa and Veslind, 2001). Before sludge being used as blocks or cement, the wet sludge cakes can be processed by drying and burning to remove moisture, followed by grounding and sieving to produce sludge powder. This process would cause energy waste and air pollution.

AD is one of the useful technologies to treat sewage sludge, generating renewable biogas (Ding et al., 2017; Iacovidou et al., 2012). Biogas comprises of 60–70% methane, and 30–40% of carbon dioxide, with a relative density of around 0.85, and calorific value of about 13–21 MJ kg^{-1}, which is lower than that of coal (15–27 MJ kg^{-1}). However, due to the nature of WWTPs, sewage sludge usually has low bioavailability and/or biodegradability (Carlsson et al., 2012, Parkin and Owen, 1986). In addition, AD of sewage sludge requires a long retention time (20–30 d) as well as a large digester volume, which eventually leads to a high capital cost (Appels et al., 2008, Han et al., 2017a).

Composting is a spontaneously biological decomposition process involving mineralization and humification of organic materials in a predominantly aerobic environment. During the process, bacteria, fungi and other microorganisms, including micro arthropods, can break down the macromolecular organic materials into usable nutrients and stable organic substances called as compost (Nasini et al., 2016), which is also free of pathogens and seeds of weeds (Eghball and Lesoing, 2000). However, the production of finished compost generally need at least 3 months by conventional composting process (Ros et al., 2003). Thus, the development of new technologies to accelerate the composting process is indispensable to meet the treatment demand for increased amount of sewage sludge.

1.2 Hyper-thermophilic aerobic compost (HTAC)

Hyper-thermophilic aerobic composting (HTAC) is one of the innovative methods that have been applied in small local communities in Japan. The bacteria which can survive at high temperature of 80-100°C, i.e. hyper-thermophile, are used during hyper-thermophilic aerobic composting.

Hyper-thermophile usually can be separated from extreme environment, such as hot spring, hot-water deposit in ocean, or oil deposit from underground. The metabolic heat release that occurs during bacterial fermentation raises the processing temperature sufficiently high to 80-100°C (Kanazawa et al., 2008). Subsequently, the composting period can be shortened to 45 days due to the high organic decomposition rate of hyper-thermophile at high temperatures. Also, a hygienic compost with better quality can be produced due to the high temperature can sterilize the pathogens. The odorous components other than ammonia also disappear in the early stage of composting process.

When the compost produced from the sewage sludge used as fertilizers, the heavy metals may also become a problem. Table 1-1 shows the heavy metals contents in HTAC and the standards in Fertilizer Regulation Act (FAMIC, 1986). The heavy metals contents in HTAC are all lower than the standards, indicating that it can be used as fertilizer safely. Meanwhile, the high concentration of phosphorus (38000 mg kg^{-1}) contained in HTAC may increase its economic benefits, as most of the phosphorus source is derived from phosphate-rich rocks, which exist only in a few countries, predominantly China, Morocco, Russia, and United States (Survey, 2013). Reuse of phosphorus from sewage sludge is a crucial issue considering that P is a finite resource (Kim et al., 2018).

1.3 The disadvantages of chemical fertilizer

Cereal accounts for about 80% of the world's food supply (Pimentel and Wilson, 2004), in which the rice provides 20% of the world dietary energy supply (FAO, 2004). With the rapid increase of world population, the quantity of food produced per capita has been declining since 1984 based on the available cereal grains (Pimentel and Wilson, 2004). Though the expansion of arable land can remit the eager increasing food demand, fertilizer still is the predominant factor with regard to the increased grain yield. It was reported that cereals account for around

60% of global fertilizer use (FAO, 2012). However, the increased application of chemicals fertilizer resulted in serious soil deterioration. The structure, exchangeable cations (especially calcium and magnesium) and pH of soil can be significantly affected by application of chemical fertilizers for months and years (Bernal et al., 2009). Cai et al. (2015) reported that based on the 18-year fertilization treatments, urea as a chemical N fertilizer in an intensive farming system has significantly reduced soil pH and is confirmed as the major cause of intensified acidification of the red soil in southern China. As a trend, the use of organic fertilizer has been paid more attention since it can improve the structure of the soil and increase its ability to hold water and nutrients (Crecchio et al., 2001; Tester, 1990).

1.4 Submerged plants

Organic fertilizer has been paid more attention since it can improve the structure of the soil and increase its ability to hold water and nutrients. Except the HTAC, there still have many kinds of organic fertilizers. 'Green manure', which has been reported by many literatures, can be produced by leaving uprooted parts of plant to wither on a field to improve the soil structure and provide nutrients for plant growth. It has already been used for hundreds of years in China and some other countries.

Submerged plants are an important component of the aquatic primary production and biogeochemical cycles. Submerged plants can reduce water turbidity caused by phytoplankton and suspended solids, and have better nitrogen (N) and phosphorus (P) removal efficiency than emergent plants. However, when submerged plants are excessively growing, they would also cause seriously environmental problems in lakes.

Ceratophyllum (C.) demersum and *Egeria densa* can grow all year around and contain high contents of P and N, which can be easily degraded. If the submerged plants could be used as organic fertilizers, the method of utilizing submerged plants to solve eutrophication in lakes will be more effectively and environmentally friendly. It can save money and time without pretreatment.

1.5 Objectives of this research

This study aimed to utilize biomass wastes as fertilizers to raise the crop yield and recycle nutrients resource appropriately. The fertilizer potentials of HTAC produced from sewage sludge by hyper-thermophilic aerobic composting and the submerged plants which has been used for nutrients absorption in lakes were evaluated as follows:

(1) Seed germination test using seeds of Komatsuna was conducted to evaluate the phytotoxicity of HTAC.

(2) Komatsuna cultivation by using soilless culture was performed to evaluate the fertilizer quality of HTAC. The characteristics of roots, leaves and stems of cultivated Komatsuna were measured to compare in each condition with different amount of HTAC addition.

(3) The two years' rice growth experiment was conducted by applying HTAC to further elucidate the optimum fertilization amount, long-term application effect and environmental impact by applying HTAC on farmland.

(4) The effect of mixture of HTAC, *C. demersum* and *Egeria densa* with nitrogen content of 180 kg N ha^{-1} on rice growth and yield were also carried out to evaluate the fertilizer potential of submerged plants.

1.6 Novelty and structure of the thesis

This study first evaluated the fertilizer potential of HTAC produced from sewage sludge by a innovative technology, HTAC and submerged plants *C. demersum* and *Egeria densa*. The optimum fertilization amount of HTAC for rice growth also was determined through two years' field experiment. Sewage sludge contains large amount of nutrients that can be recovered and used for crop growth. HTAC manufacturing process could produce organic fertilizer from sewage sludge during a shorter period. However, there is no research report on the safety and quality of the compost produced through this process. Compared with the chemical fertilizers, HTAC contains not only mineralized nutrients, but also organic nutrients that can be slowly decomposed to release nutrients for plant growth. Overdosing would induce environmental pollution and long-lived phenomena in rice crops and infect the rice yield. Thus, it is also necessary to determine the fertilization amount of HTAC. Except for sewage sludge that is generated during wastewater treatment processes, excessive growth of submerged plants, which could reduce water turbidity in lakes caused by phytoplankton and suspended solids, and have excellent nutrients removal efficiency, also has become a serious problem for maintaining the ecological balance in lakes. The nutrients absorbed by submerged plants could be further recycled and reutilized, to solve eutrophication in lakes, which could provide a more sustainable solution. To achieve these objectives, the thesis was divided into five chapters:

Chapter 1 Introduction

The current status of studies on organic fertilizers was introduced. Then the objective and structure of the thesis were addressed.

Chapter 2 Seed germination test and Komatsuna cultivation by applying the HTAC

In order to investigate the stability and maturity of HTAC, seed germination test and Komatsuna cultivation without soil were conducted. Germination index (GI) value during seed germination test was calculated to indicate the phytotoxicity of HTAC. For soilless cultivation of Komatsuna, the characteristics of roots, stems and leaves of cultivated Komatsuna were measured to indicate the quality of HTAC. Komatsuna was chosen in this study because of the following two reasons: 1) Komatsuna is a variety of *Brassica Rapa*, related to Chinese cabbage, which has been reported as the most sensitive seeds to indicate the maturity of compost; 2) Due to its character of fast growth, it is more suitable to be used as experimental samples in the laboratory.

Chapter 3 Optimum dosage of hyper-thermophilic aerobic compost produced from sewage sludge for rice growth and yield

According to one year's experiment of rice cultivation, optimum dosage of HTAC was clarified by comparing stem height, tillers number and rice yields. N and P concentrations in the surface water during rice cultivation were detected to evaluate environmental friendliness when applying HTAC on farmland. Rice is one of the most popular cereal in the world. If HTAC could be successfully used for enhancing rice yield, its contribution would be significant.

Chapter 4 Hyper-thermophilic aerobic compost and submerged plants for rice growth and yield

HTAC was further applied for the second year in order to elucidate the long-term application effect. In addition, the applicability of the mixture of HTAC, *C. demersum* and *Egeria densa* with nitrogen content of 180 kg N ha^{-1} (optimum dosage concluded in Chapter 3) at different ratios were analyzed. Since the fertilizer potential of HTAC has been proven in Chapter 2 and Chapter 3, the fertilizer potential of submerged plants was investigated by mixing with HTAC at the optimum fertilization dosage that determined in Chapter 3.

Chapter 5 Conclusions and future researches

The results from Chapters 2-4 were concisely concluded, and future researches were prospected.

Table 1-1 The nutrients and heavy metals contents in the hyper-thermophilic aerobic compost (HTAC) and their standards in Fertilizer Regulation Act*.

Parameters (units)		HTAC	Standard in Fertilizer Regulation Act
TS	(%)	73.5	-
TN	(mg kg^{-1})	28000	-
NH_4^+-N	(mg kg^{-1})	20000	-
TP	(mg kg^{-1})	38000	-
As	(mg kg^{-1})	2.6	< 50
Cd	(mg kg^{-1})	0.9	< 5
Hg	(mg kg^{-1})	0.4	< 2
Ni	(mg kg^{-1})	19	< 300
Cr	(mg kg^{-1})	22	< 500
Pb	(mg kg^{-1})	12	< 100

*Source: Food and Agricultural Materials Inspection center (FAMIC), 1986. Fertilizer Regulation Act.

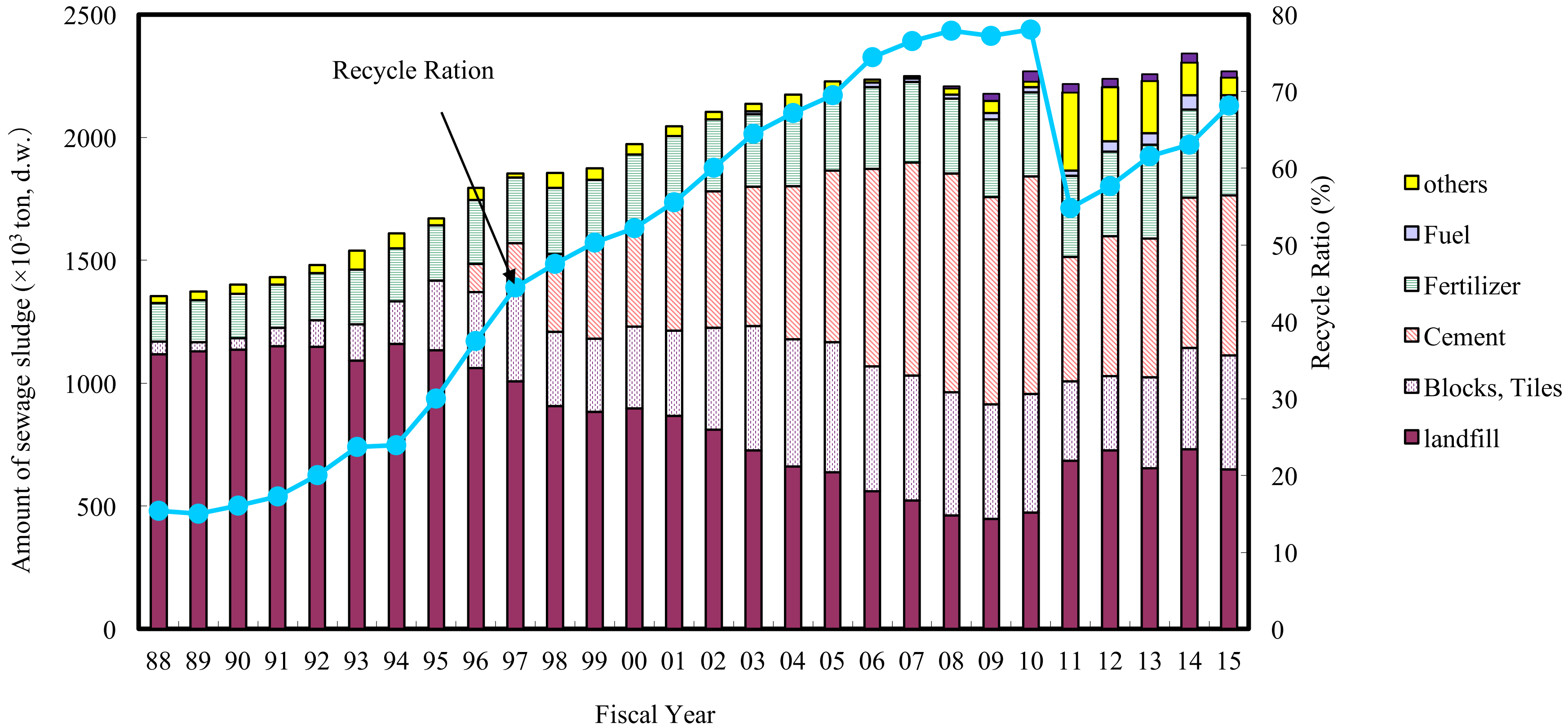


Fig. 1-1 Promoting the material use of sewage sludge.

*Landfill and other types of disposal increased in FY2011 as a result of the Great East Japan Earthquake.

Source: Ministry of Land, Infrastructure, Transport and Tourism (MLIT), available at:

http://www.mlit.go.jp/mizukokudo/sewerage/crd_sewerage_tk_000124.html

Chapter 2 Seed germination test and Komatsuna cultivation by applying HTAC

2.1 Introduction

Compost quality, such as stability and maturity, should be confirmed before applying on farmland. The unstable and/or immature compost can result in adverse effects on seed germination, plant growth and soil environment due to the decreased supply of oxygen and/or available nitrogen or the presence of phytotoxic compounds (Bernal et al., 2009).

In this chapter, Komatsuna was used for seed germination test. Emino and Warman (2004) compared the germination index (GI) values of mature and immature municipal solid waste compost with three groups of seeds that included the large size (green bean, sweet corn, hybrid cucumber and sunflower), medium size (broccoli, Chinese cabbage, radish, tomato,) and small size (cress, carrot, lettuce and petunia) seeds. They concluded that most of the species, including cress seed, were not sensitive enough to discriminate mature and immature compost, while Chinese cabbage seed was the most sensitive one. Komatsuna, a variety of *Brassica Rapa*, is related to Chinese cabbage. Besides, it is one of the most common vegetables in Japan. Thus, Komatsuna was selected for seed germination test in this study.

Soilless cultivation is intensively used in agriculture to improve the management over growing conditions for plants and to avoid uncertainties of nutrients status in the water and soil. The advantages of soilless culture are: absence of soil-borne pathogens; safe alternative to soil disinfection; nutrients and water are applied more evenly to the plants (Putra and Yuliando, 2015). HTAC contains high level of nutrients, such as phosphorus, ammonia nitrogen, and potassium. Through hyper-thermophilic composting and soilless cultivation, the nutrients in sewage sludge could be utilized efficiently. Furthermore, plant growth assay using soilless cultivation can reflect the fertility and applicability of HTAC more clearly by eliminating the effect of microbes in soil.

There is no research published on soilless cultivation with HTAC up to now. Thus, this study aims to investigate the fertilizer potential and feasibility of HTAC applying in soilless culture.

2.2 Materials and methods

2.2.1 Seed germination

(1) Compost extraction

HTAC used in this research was provided by Kyowa Kako Co., Ltd. It was produced by composting sewage sludge inoculated with *C. yamamurae*, which was isolated from Kirishima hot spring in Kagoshima prefecture, Japan. The sewage sludge was collected from Kagoshima wastewater treatment plant. The main characteristics of HTAC are shown in Table 2-1.

Seed germination test was carried out in University of Tsukuba. 4 g HTAC was mixed with 40 mL distilled water. Then the sample was centrifuged for 15 min at 9900 r min^{-1}. The electrical conductivity (EC) of compost extraction filtrate was 5.8 mS cm^{-1}. Thus, the filtrate was diluted correspondingly to EC of 5.8 (R2), 2.9 (R3),1.5 (R4) or 0.7 mS cm^{-1} (R5). R1 which using distilled water, was used as control.

(2) Seed germination test

The seeds of Komatsuna were chosen for this germination test. For each test, 1.5 ml filtrate or distilled water was added on the filter paper in a petri dish where 20 seeds were placed. Two petri dishes were prepared for each condition. Then, the seed germination test was conducted at 28°C and 60% of humidity. After 72 hours' incubation, the number of viable seeds and the root length in each test were measured and recorded. Besides, the electric conductivity (EC) of each filtrate used for germination test were measured. The seed germination (SG), relative seed germination (RSG), relative radicle growth (RRG) and seed germination index (GI) were calculated using the following equations (Luo et al., 2018).

$$SG=\frac{Q_g}{Q_t}\times 100\% \quad (2.1)$$

$$RSG=\frac{Q_{gs}}{Q_{gc}}\times 100\% \quad (2.2)$$

$$RRG=\frac{L_{gs}}{L_{gc}}\times 100\% \quad (2.3)$$

$$GI=RSG\times RRG\times 100\% \quad (2.4)$$

where Q_g is the number of germinated seed, and Q_t is the number of total seeds. Q_{gs} and Q_{gc} are the number of germinated seed in the tested filtrate sample and that in the control (distilled water). L_{gs} and L_{gc} are the average radicle length of germinated seeds in the tested filtrate sample and those in the control, respectively.

(3) Statistical analysis

Pearson's correlation coefficients were calculated to show the relationship between GI and chemical properties of HTAC extraction at different nitrogen concentration levels. All the statistical analyses were conducted by using IBM SPSS Statistics 20.0.

2.2.2 Komatsuna cultivation by applying the HTAC in soilless culture system

(1) Experimental setup

As shown in Fig. 2-1, 500 mL white plastic bottles were used as culture containers. On the top of each bottle, sponge was located to maintain the Komatsuna seedling.

(2) Seed sprouting

Before cultivation, 100 seeds were sprouted together in an incubator at 28°C for one week. After coming up 2 cotyledons, 10 Komatsuna seedlings that had the same radical length were displaced in the container.

In each container 500 mL distilled water was added. 1.33 g (B1), 2.66 g (B2), 5.32 g (B3) and 8.47 g (B4) of HTAC was introduced into each bottle. Two bottles were prepared for each concentration. The treatment with no fertilizer was used as the control. Then the Komatsuna were cultivated for one month in a black box. The light was provided from 6 am to 10 pm everyday artificially.

(3) Calculation of yield components

The Komatsuna length was measured by the length of stem from the root to the longest leaf. The root length, stem length, maximum leaf length and width of Komatsuna were measured after 30 days' cultivation. The longest stem length and total number of leaves per plant in each condition were also recorded.

After harvesting Komatsuna, the fresh weight of root, stem and leaf were measured. Then Komatsuna was dried for 72 h at 60°C and the dry weight was measured.

(4) Statistics

Analysis of variance was conducted to detect the differences in length and weight. Data were analyzed in factorial in randomized complete block design. The least significant difference (LSD, Tukey-Kramer) at $p < 0.05$ was applied to compare the means for significant differences between the various conditions.

2.3 Results and discussion

2.3.1 Germination test

(1) Germination rate and length of root and stem of Komatsuna

The length of root and stem and seed germination (SG) are shown in Fig. 2-2. The germination rate in R1 (distilled water) was 85%, which is more than 70%, indicating that the seed selected was desirable for the test (Luo et al., 2018). Compared to the control (R1), the germination rate in R2-R5, with filtrate of HTAC were increased by 14.7%, 17.6%, 8.8% and 8.8%, respectively. However, the root length was decreased by 41.6%, 20.5%, 32.2%, 54.3%, respectively, while the stem length was increased by 67.7%, 115.4%, 112.1%, and 38.8%, respectively. R1 had the longest root length, while R3 had the longest stem length and highest germination rate. The total length of root and stem in R3 was also greater than that in R1. This proved that the HTAC not only has no phytotoxicity, but also can improve the elongation of plant stem.

(2) Germination index (GI)

Seed germination can be used to indicate the high toxicity of compost, and radicle growth can be used to examine the compost with low toxicity (Zucconi et al., 1981; Tiquia et al., 1996). Thus, GI value combining these two factors can be effectively used as an indicator for phytotoxicity of fertilizers.

The GI value, not less than 80%, usually means that compost has no phytotoxicity (Tiquia et al., 1996). From Table 2-2, it can be seen that only in R3, the GI value was higher than 80%, while the others were all lower than 80%. Thus, HTAC had no phytotoxicity when it was diluted by 20 times (EC=2.9 mS cm^{-1}). This indicated that the dilution time was also an important factor affecting the phytotoxicity of compost.

Moreover, the time courses of seed germination usually took several days under suitable conditions, which can be morphologically divided into three phases, consisting of phase I (imbibition), phase II (radicle emergence) and phase III (radicle elongation). The uptake of water is the major process of seed germination during phase I, which could be negatively affected by high salinity of compost. During phase II, the low molecular weight organic acids in compost could be the primary inhibitor of radicle emergence after testa rupture. Seen from Table 2-2, R2-R5 had higher RSG (over 100%) compared with the control (R1). So there was no inhibition occurred during phase I (imbibition) and phase II (radicle emergence). The salinity and low molecular weight organic acids in HTAC were not the inhibitors. Radicle elongation could be inhibited by NH_4^+ during the phase III (Luo et al., 2018). RRG in R2-R5 were lower compared with the control (R1). This might be due to the inhibition effect of high NH_4^+ concentration in HTAC.

Although it is known that the growth of shoot and root can be adversely affected by ammonia toxicity, the present study indicated that root is more sensitive to ammonia toxicity than shoot (Wan et al., 2016). In some research, the concentration of ammonium is used as an indicator to evaluate the maturity of compost (Ko et al., 2008). The reason is that nitrification is generally involved in the composting process. Nitrifying bacteria can convert ammonium into nitrate by nitrification process. However, during the HTAC manufacturing process, nitrifying bacteria cannot survive because of the high fermentation temperature (80-100°C). Thus, nitrification cannot happen during hyper-thermophilic aerobic composting (Kanazawa et al. 2003). This does not mean that HTAC is immature because the ammonium nitrogen also is bioavailable. However, more attention should be paid on ammonium toxicity when applying HTAC on farmland.

2.3.2 Komatsuna cultivation

(1) The leaf and root character of Komatsuna

The number and length of leaves of Komatsuna after one month's cultivation are shown in Table 2-3. Although B4 had the maximum number of leaves, there was no significant difference between the different treatments. While the total length of leaves was significantly longer in B3 and B4. The control without fertilizer did not grow normally. With the increase of compost amount, the total length of leaves increased firstly and then decreased. In B4, there was no leaf longer than 25 cm, but there were too many leaves with length between 10 to 20 cm. With the amount of HTAC increasing, the number of leaves increased, but the length of longest leaf decreased (B4). Maybe the high concentration of some substance in HTAC in B4 inhibited the growth of Komatsuna.

Table 2-4 shows the longest length of root and leaf. In B1, the root of Komatsuna was the longest. With the amount of fertilizer increasing, the length of root decreased. In B1, the concentration of ammonium nitrogen was lower than B2-B4. The results in seed germination test indicated that ammonium nitrogen might limit the elongation of radicle. During Komatsuna cultivation, ammonium nitrogen might also inhibit the root elongation. Compared with B1, B3 had the shorter root length, but had longer stem and leaf length. Thus, the growth of Komatsuna was not affected, even the root length was shorter in B3.

(2) The weight of root and stem of Komatsuna

The fresh weight, dry weight and moisture content of root and stem are shown in Table 2-5. The fresh weight of root and stem in B3 were significantly higher than that in B1, B2, and B4. The fresh weight of stem in R3 increased by 122.91%, 59.05%, 33.09%, respectively when compared with that in B1, B2 and B4. Although the longest root length in B3 was shorter than B1 and B2, the fresh weight of root in B3 was significantly higher than others. It means that in B3 there had more roots to absorb water and nutrients for Komatsuna growth. Thus, 5.32 g HTAC addition in 500 mL water not only increased the yield of Komatsuna, but also increased the weight of root.

In this study, the yield in B3 was the highest. Compared with the normal yield, however, the result was still lower. In the future study, other factors, such as light intensity, temperature and humidity, should be further considered in soilless culture system.

2.4 Summary

The maturity and phytotoxicity of HTAC was tested by seed germination test. The results indicated that HTAC had no phytotoxicity, which can also enhance the seed growth. However, more attention should be paid to ammonium toxicity when applying HTAC on farmland.

HTAC was also applied for Komatsuna cultivation in soilless culture system. It was successfully used as fertilizer in the soilless culture system. In this study, the optimum dosage was determined as 5.73 g/500 mL in the soilless culture system for Komatsuna cultivation.

Table 2-1 Characteristics of HTAC.

Parameters (units)		HTAC
TS	(%)	73.5
TN	(mg kg^{-1})	28000
NH_4^+-N	(mg kg^{-1})	20000
TP	(mg kg^{-1})	38000
C/N ratio		6
TK	(mg kg^{-1})	8100
Cu	(mg kg^{-1})	250
Zn	(mg kg^{-1})	470
CaO	(mg kg^{-1})	70000

*TS, total solids content; TN, total nitrogen content; TP, total phosphorus content; TK, total potassium.

**The data were measured by Syonan analysis center Co., Ltd.

Table 2-2 The germination index of compost extract in different conditions.

	RSG (%)	RRG (%)	GI (%)
R2	115	59	68
R3	118	79	94
R4	109	68	74
R5	109	46	49

RSG, relative seed germination; RRG, relative radicle growth; GI, seed germination index. The calculation could refer to equation (2.2-2.4) in section 2.2.1 (2).

R1, control, distilled water; R2, 10 times of dilution of compost extract filtrate; R3, 20 times of dilution of compost extract filtrate; R4, 40 times of dilution of compost extract filtrate; R5, 80 times of dilution of compost extract filtrate,

Table 2-3 Number and length of leaves of Komatsuna after one month' cultivation in soilless culture system.

	<5 cm	<10 cm	<15 cm	<20 cm	<25 cm	Total number	Total length (cm)
B1	4.5 ± 0.5	0.5 ± 0.5	2.0 ± 1.0	3.0 ± 1.0	0.0	10.0 ± 1.0[a]	102.5 ± 1.0[a]
B2	4.0 ± 0.0	2.0 ± 0.0	1.0 ± 0.0	3.5 ± 0.5	1.0 ± 0.0	11.5 ± 0.5[a]	132.8 ± 2.3[b]
B3	3.5 ± 0.5	2.0 ± 0.0	2.0 ± 1.0	2.5 ± 1.5	2.0 ± 0.0	12.0 ± 1.0[a]	149.5 ± 4.0[c]
B4	4.5 ± 0.5	1.5 ± 0.0	4.0 ± 2.0	3.0 ± 1.0	0.0	13.0 ± 1.0[a]	138.0 ± 4.5[bc]

Data were statistically analyzed by one-way ANOVA with repeated measures and different small letters indicate significant differences ($p < 0.05$) among four treatments of each season by Duncan's method for multiple comparisons. All the data are the means of two replicates ± standard error.

Table 2-4 The length of root, stem and leaf and the width of leaf in different conditions (the maximum length and width were recorded).

	Root (cm)	Stem (cm)	Leaf length (cm)	Leaf width (cm)
B1	25.50 ± 4.50^{c}	16.35 ± 0.85^{a}	7.70 ± 0.10^{a}	4.35 ± 0.15^{a}
B2	20.00 ± 4.00^{b}	20.50 ± 0.00^{b}	10.25 ± 0.25^{b}	5.35 ± 0.15^{b}
B3	19.75 ± 1.25^{b}	21.45 ± 0.05^{b}	10.35 ± 0.15^{b}	6.65 ± 0.45^{c}
B4	17.00 ± 0.00^{a}	18.15 ± 1.65^{a}	10.10 ± 0.40^{b}	5.50 ± 1.00^{b}

Data were statistically analyzed by one-way ANOVA with repeated measures and different small letters indicate significant differences ($p < 0.05$) among four treatments of each season by Duncan's method for multiple comparisons. All the data are the means of two replicates ± standard error.

Table 2-5 The fresh weight, dry weight and moisture content of root and stem in different conditions.

	Weight (g)	Dry weight (g)	Moisture content (%)
Root			
B1	1.23 ± 0.26 ^{a}	0.08 ± 0.01 ^{a}	93.19 ± 0.42 ^{a}
B2	0.92 ± 0.11 ^{a}	0.08 ± 0.01 ^{a}	91.30 ± 2.41 ^{a}
B3	4.6 ± 0.48 ^{b}	0.25 ± 0.06 ^{a}	94.67 ± 0.66 ^{a}
B4	1.89 ± 0.95 ^{a}	0.13 ± 0.07 ^{a}	92.97 ± 0.08 ^{a}
Stem			
B1	4.06 ± 0.28 ^{a}	0.47 ± 0.13 ^{ab}	89.00 ± 2.43 ^{a}
B2	5.69 ± 0.19 ^{b}	0.39 ± 0.01 ^{a}	93.00 ± 0.40 ^{a}
B3	9.05 ± 0.67 ^{c}	0.85 ± 0.07 ^{b}	91.00 ± 0.02 ^{a}
B4	6.8 ± 0.2 ^{b}	0.55 ± 0.13 ^{ab}	92.0 1.60 ^{a}

Data were statistically analyzed by one-way ANOVA with repeated measures and different small letters indicate significant differences ($p < 0.05$) among four treatments of each season by Duncan's method for multiple comparisons. All the data are the means of two replicates ± standard error.


Fig. 2-1 The image of soilless culture system used for Komatsuna cultivation.

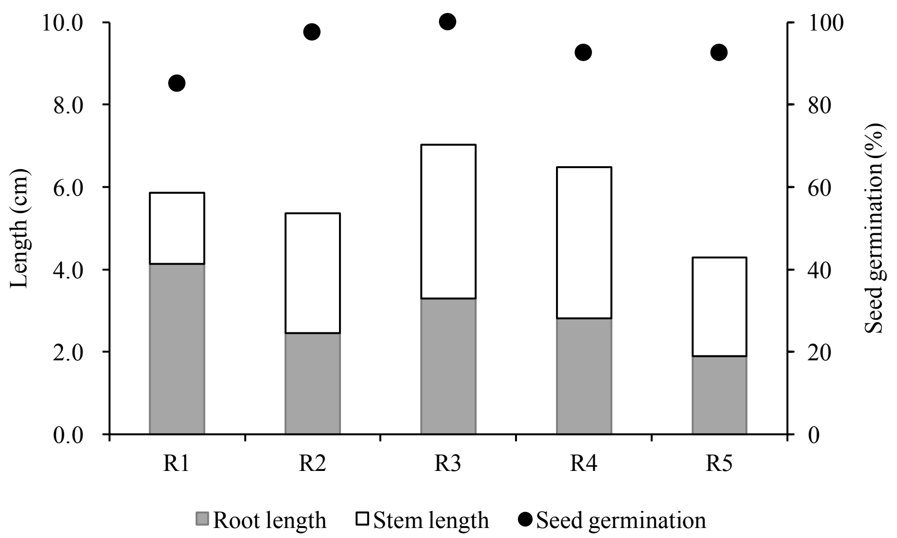


Fig. 2-2 The seed germination and length of root and stem during seed germination test.

Chapter 3 Optimum dosage of HTAC produced from sewage sludge for rice growth and yield

3.1 Introduction

Long-term application of chemical fertilizers shows negative effect on soil's N and C storage capacities and agronomic efficiency (Khan et al., 2007; Mulvaney et al., 2009). In addition, the production of synthetic fertilizer requires a large amount of gases, energy (Haber-Bosch process for N) and natural mineral resources (like phosphate rock) (Teenstra et al., 2014).

The compost produced from hyper-thermophilic aerobic composting could have better quality since the high temperature can sterilize the pathogens. The odorous components other than ammonia also disappear in the early stage of fermentation process.

The results from chapter 2 showed that HTAC has no phytotoxicity, and it also can improve the seed growth. However, more attention should be paid to ammonium toxicity when applying HTAC on farmland. However, compared with chemical fertilizers, HTAC contains not only mineralized nutrients, but also organic nutrients that can be slowly decomposed to release nutrients for plant growth. Thus, it is important to determine the fertilization amount of compost since overdosing would induce environmental pollution and long-lived phenomena in rice crops, and impact the rice yield.

In this study, a HTAC produced in Kagoshima, Japan was applied to evaluate the effects on rice growth and environmental burden derived from rice paddy drainage. The rice growth experiment was carried out in 2016. A rice cultivar called as Koshihikari was chosen for the plant growth experiment. This study aimed to elucidate the optimum application amount of HTAC for rice growth.

3.2 Materials and methods

3.2.1 Fertilizers

HTAC used in this research was provided by Kyowa Kako Co., Ltd. It was produced by composting sewage sludge inoculated with C. *yamamurae*, which was isolated from Kirishima hot spring in Kagoshima prefecture, Japan. The sewage sludge was collected from Kagoshima wastewater treatment plant (Moriya et al., 2011). The main characteristics of HTAC are shown in Table 2-1.

The chemical fertilizer used in this study was manufactured by Showa Sangyo Co., Ltd. It has a N: P: K ratio of 14.0: 14.0: 14.0.

3.2.2 Study site and rice growth experiment

The rice growth experiment was performed on a farmland in Tsukuba, Ibaraki, Japan (36°05′N,140°04′) in 2016. This region has a northern temperate marine climate with an average annual temperature of 13.8°C and a mean annual precipitation of 1283 mm.

Square containers (0.8 m in length × 0.5 m in width × 0.5 m in height) equipped with water inlet and outlet were used as rice growth device to evaluate the different fertilization condition (Fig. 3-1a). In each container, commercial pumice and river sand were firstly filled for 5 cm orderly from the bottom. Then, the well mixed paddy soil was filled on top for 30 cm (Fig. 3-1b). A specific amount of HTAC was applied in each container and mixed well with the paddy soil. The experiment without fertilizer addition was used as the control. After irrigating, 8 rice plants (cultivar: koshihikari) were transplanted in each device with the interval of 20 cm. The devices were placed outdoors. The rice growth period was from May 5, 2016 to September 12, 2016. The fertilization conditions are shown in Table 3-1. Mid-summer drainage was conducted between days 44 and 63 during rice growth experiment as it was proven that summer drainage could enhance rice growth and yield (Hwang and Chung, 2013).

3.2.3 Calculation of rice yield and yield components

The plant height was measured by the length of rice plant from ground to the longest leaf. Tillers per plant was recorded by the total number of stem that grew after the initial parent stems.

After rice harvest, the rice plants were air-dried for two weeks. The numbers of panicle and spikelet were counted. The ripened grains were obtained by salt water selection method using NaCl solution with a specific gravity of 1.06. The ripened grains were then cleaned by distilled water and air dried for 24 h. The outer hull of ripened grains was removed by hulling machine (TR-130, manufactured by KETT electric laboratory). The weight of rice per plant after removing the hull was called as brown rice weight (g plant^{-1}). The ripening rate of grain, 1000-grain weight and rice yield were calculated by the following equations.

$$\text{Ripening rate of grain (\%)} = \frac{\text{number of ripened grains}}{\text{spikelets per plant}} \times 100 \quad (3\text{-}1)$$

$$\text{1000-grain weight (g)} = \frac{\text{brown rice weight}}{\text{spikelets per plant} \times \text{ripening rate of grain}} \times 1000 \quad (3\text{-}2)$$

$$\text{Rice yield (t ha}^{-1}) = \frac{\text{brown rice weight} \times 8 \text{ (plant number per device)}}{0.4 \text{ m}^2 \text{(cross sectional area of the device)}} \times 10000 \quad (3\text{-}3)$$

3.2.4 Determination of N and P in surface water and soil

Surface water samples were collected from each device every week. The water samples were filtered through fiber filter with a pore size of 1.2 μm. Then, the concentration of total nitrogen (TN), NH_4^+-N, NO_3^--N, and total phosphorus (TP) in all the water samples were determined. TN was determined by alkaline potassium persulfate digestion and UV spectrophotometric method (APHA, 2012). NH_4^+-N was measured by Indophenol-blue method (Ivancic and Degobbis, 1984). NO_3^--N was measured by UV spectrophotometer method at 220 and 275 nm (APHA, 2012). TP was determined by a molybdenum blue method modified by Murphy and Riley (1962).

In each device, soil was randomly sampled from five spots at a depth of 0-20 cm. Wet soil samples were air dried, grounded and passed through 2 mm sieve. NH_4^+-N in soil was measured by the indophenol blue method proposed by Hall (1993). PO_4^{3-}-P in soil was determined by the molybdenum blue colorimetric method after extraction by 0.5 M $NaHCO_3$ (Olsen, 1954).

3.2.5 Statistics

Analysis of variance was conducted to detect differences in plant height, rice yield, and yield components. Data were analyzed in randomized block design. The least significant difference (Duncan method) at $p < 0.05$ was applied to compare the means for significant differences between variety and cropping season.

3.3 Results and discussion

3.3.1 Characteristics of rice growth

(1) Plant height

The variation of plant height over time is shown in Fig. 3-3a. Compared with R2 (Che. 60), R4 (HTAC 180) showed a similar level of plant height, while a higher plant height was achieved in R5 (HTAC 250) and R6 (HTAC 500). The highest plant height reached to 105.3 cm in R6 (HTAC 500) after 91 days' cultivation and began to flatten after that. As shown in Fig. 3-3b, on day 115, plant height was found to be positively correlated with the fertilization amount of HTAC. The relationship between plant height and HTAC fertilization amount fitted the following quadratic equation: y =0.0343x+90.604 (R^2= 0.979; x, HTAC amount; y, plant height).

The average height in panicle formation stage and maturity stage are shown in Table 3-2. The plant height increased with increasing N fertilization amount in both stage in R3-R6. And R4 (HTAC 180) showed a similar plant height with R2 (Che. 60).

Rice lodging occurred on rice plant in R6 (HTAC 500) with the highest amount of HTAC. It can easily happen when the stem over grew. The normal height of Koshihikari is said to be less than 80 cm in panicle formation stage, and less than 100 cm in maturity stage. The height of plant is an important indicator related with rice lodging. From Table 3-2, we could found that the plant in R4-R6 showed higher plant height than normal value. Basak et al. (1962) reported that high nitrogen fertilization amount might lead to rice lodging and pointed out that 100 pounds per acre (112 kg N ha^{-1}) gave optimum yield of rice and avoided rice lodging before heading.

(2) Rice tillers per plant

The variation of tillers per plant are shown in Fig. 3-4a. After transplanting, the number of stem increased with the occurrence of tiller and reached the maximum value. Then the ineffective tillers died during the summer drainage, and panicle grew from the remained tillers. The tillers number in all the treatments reached the highest on day 49, which were 10, 27, 14, 24, 26, 35 in R1 to R6, respectively. Among all the treatments, R6 (HTAC 500) achieved the highest tillers number.

As seen in Fig 3-4b, on day 49 and day 115, tillers number were found to be positively correlated with the fertilization amount of HTAC. The relationship between tillers number and HTAC amount fitted the following quadratic equations: y =0.0521x+11.385 (R^2= 0.955; day 49; x, HTAC amount; y, tillers number), y=0.0462x+9.0525 (R^2= 0.994; day 115; x, HTAC amount; y, tillers number).

In this research, the rice growth was normal after transplanting under all the treatment conditions. There was no death or growth defect happened in these treatments. Compost has been recognized as a slow-releasing fertilizer, but the maximum tillers number and panicle formation stage happened at the same time under both chemical fertilizer and HTAC application. This might be due to the high ammonium concentration in HTAC that can provide sufficient nutrients for rice growth.

3.3.2 Yield and yield components

As shown in Table 3-3, the rice yields in R3-R6 significantly increased with the increase in the fertilization amount of HTAC. The highest rice yield was 6.56 t ha^{-1} in R6 (HTAC 500). In R3-R6, the rice tillers number and spikelets number per plant also increased with the increasing fertilization amount, while the ripening rate of grain and 1000-grain weight decreased when HTAC amount higher than 250 kg N ha^{-1}.

Under these experimental conditions, the relationship between rice yield and HTAC fertilization amount fitted the following quadratic equation: $y = -1.858\times10^{-5}x^2+0.017x+2.667$ (R^2= 0.968), and the optimal fertilization amount calculated was 457.5 kg N ha^{-1} (Fig. 3-5a). As the rice yield of R2 (Che. 60) was 5.02 t ha^{-1}, the amount of HTAC which can achieve the same rice yield with R2 calculated by this modified quadratic equation is 170.0 kg N ha^{-1}.

The higher fertilization amount of HTAC increased the risk of rice lodging, which might also influence the rice yield. Fig. 3-5b shows the relationship between rice yield and tillers number. When the tillers number was lower than 22, the relationship between tillers number and rice yield fitted the following quadratic equation: $y = 0.2658x+0.3913$ (R^2= 0.994; x, tillers number; y, rice yield). However, in R6, although the tillers number reached to 35, the rice yield did not increase significantly.

The rice yield in R6 (HTAC 500) was the highest (6.56 t ha^{-1}), which is 2.24 times that in R1 (NONE). However, the ripening rate of grain was found to be lower in R5 and R6. The higher nutrients level applied in the soil can promote plant growth, thus increasing the tiller number per plant. The more tillers can guarantee the sufficient photosynthesis for plant growth, and in turn, can potentially increase the rice yield. However, when the tiller number excess a specific value, malnutrition would be happened. The spikelet number per panicle would decrease due to the malnutrition. However, in this research, spikelet number per panicle in all treatments had no significant difference. That is, the rice yield increased with the increase of tiller number. The NH_4^+-N and PO_4^{3-}-P concentrations in soil were relatively higher in R5 (HTAC 250), R6 (HTAC 500) (Fig. 3-6). Thus, enough nutrients were supplied to promote the spikelet growth on each panicle, though lower ripening rate of grain was observed in R5 and R6.

The rice growth in R3 (HTAC 60) was inferior compared with that in R2 (Che. 60), which was applied with the same amount of nitrogen. While the rice growth in R4 (HTAC 180) showed a similar level with that in R2 (Che. 60). Compost contains abundant organic matters. The N exiting forms in HTAC include both inorganics and organics, while chemical fertilizer only contains inorganic N. As we known, inorganic N (NH_4^+-N and NO_3^--N) can be directly absorbed by plant root. Though many researches demonstrate that some plants can use soluble organic N directly, but the N uptake rate of organic N is lower than that of inorganic N (Franklin et al., 2017). Most of organic N need to be firstly decomposed by microorganisms in soil and then taken up by plant as inorganic form. This might can explain why the rice growth was inferior when applying the same amount of HTAC as the chemical fertilizer. However, this does not mean that the organic fertilizer is inferior to the chemical one. Franklin et al. (2017) reported that nitrogen use efficiency can be significantly improved by applying organic N. To demonstrate this statement, the second-year experiment for rice growth was conducted. The optimized fertilization amount was 457.5 kg N ha^{-1} by calculating from the modified quadratic equation. Considering both plant quality and rice yield, 180 kg N ha^{-1}, which gives 1.84 times (5.38 t ha^{-1}) higher rice yield compared with R0 (NONE, 2.93 t ha^{-1}), was determined as the optimal fertilization amount of HTAC for rice growth. According to the Ministry of Agriculture, Forestry and Fisheries of Japan, the average rice yield in Ibaraki prefecture is 5.16 t ha^{-1} (MAFF, 2016), which is similar with rice yield at the optimal condition obtained in this study. Zhao et al. (2016) investigated four conditions with a same level of N (300 kg N ha^{-1}) for rice planting: 1) control without fertilizer; 2) chemical fertilizer (conventional dosage); 3) 50% NPK fertilizer plus 6000 kg ha^{-1} pig manure; 4) 30% NPK fertilizer plus 3600 kg ha^{-1} pig manure organic-inorganic compound fertilizer. The rice yields in conditions 2), 3) and 4) were 1.45, 1.47 and 1.54 times higher than that in control, respectively. Higher increase of rice yield (1.84 times) was achieved in this study by using only HTAC of 180 kg N ha^{-1}, indicating the good quality of HTAC for rice growth.

Table 3-5 shows the summary of composts on rice yields (selected data). Sarwar et al. (2007) found that grain yield increased significantly when the sole chemical fertilizer was applied to rice crop and in combination with compost at two levels. Minimum yield 2.41 t ha^{-1} of paddy was noted in the control while maximum 3.94 t ha^{-1} in treatment were obtained when 24 t ha^{-1} of compost was added along with chemical fertilizer. Kavitha and Subramanian (2007) studied the effect of enriched municipal solid waste compost application on growth and yield of rice in India. Dada et al. (2014) found that the grain yield (5.45 t ha^{-1}) was the highest augmented with cattle dung and maize stover compost. Hossen et al. (2016) got the highest grain yield (5.8 t ha^{-1}) by using 20 t ha^{-1}. Watanabe et al. (2017) studied the effects of rice straw composts on rice yield in Vietnam and found that the rice yields for the F40C+ and F60C+treatments were significantly higher than those from other treatments. Tejada and

Gonzalez (2006) claimed that the rice yield was influenced by applying an organic waste (crushed cotton gin compost, CC). The application of CC and inorganic fertilizers in soils increased the rice yield (5%) with respect to the application of CC without inorganic fertilizers in soils. The highest rice yield was only 0.9946 t ha^{-1}. Singh and Agrawal (2010) conducted a field study to assess the suitability of sewage sludge amendment in soil for rice by evaluating the growth and yield responses of plants grown at 0, 3, 4.5, 6, 9, 12 kg m^{-2} of sewage sludge amendment (SSA) rate. Yield of rice increased by 60%, 111%, 125%, 134% and 137% at 3, 4.5, 6, 9 and 12 kg m^{-2} of SSA, respectively, as compared to those grown in control treatment.

Among all the experiments, the rice used the HTAC had the highest rice yield. Although the weather and species in each experiment was different, it can also be confirmed that HTAC is better for rice growth.

3.3.3 Variations of N and P nutrients in water and soil

(1) Variations of N and P in water

TN, NH_4^+-N, NO_3^--N, and TP concentrations in surface water in each condition are shown in Fig. 3-6. The TN concentration increased after transplanting and reached to the highest on day 7. The highest TN happened in R2 (Che. 60, 3.89 mg L^{-1}), followed by R6 (HTAC 500, 3.34 mg L^{-1}) and R5 (HTAC 250, 3.03 mg L^{-1}). After day 7, the TN concentration of all treatments rapidly decreased. Before midsummer drainage (day 44 to day 63), they were all lower than 2 mg L^{-1}. After midsummer drainage, the TN concentration in surface water was lower than 1 mg L^{-1} in all the treatments.

The NH_4^+-N concentration in surface water showed the similar trends with the TN concentration. The NH_4^+-N concentration in R6 (HTAC 500) was the highest on day 14, increasing to 0.27 mg L^{-1}. After that, the NH_4^+-N concentration in all the treatments were maintained at concentrations lower than 0.2 mg L^{-1}. The NO_3^--N only appeared in R2 (Che. 60) in the first 7 days. It was 0.68 mg L^{-1} after transplanting and then increased to 1.83 mg L^{-1} on day 7. The NO_3^--N concentration in other treatments were all lower than 0.05 mg L^{-1}.

The variation of TP concentration was similar with the variation of TN. The TP concentration in all the treatments increased during the first 7 days after transplanting. On day 7, the highest TP concentration was 0.53 mg L^{-1} in R6 (HTAC 500). Then the TP concentrations in all the treatments decreased. Before midsummer drainage, they were all lower than 0.2 mg L^{-1}.

According to the environmental quality standard values for water in lakes and marshes

(MOE, 2016), the TN and TP in effluent water should be lower than 1 mg L^{-1} and 0.1 mg L^{-1}, respectively. The initial TN concentration in the surface water rapidly increased due to the simultaneous dissolution by irrigation. Then, it decreased by the uptake of floating plants and rice, or by leaching into the soil. The initial TN concentrations in the surface water of R2 (Che. 60), R5 (HTAC 250), R6 (HTAC 500) were relatively high (Fig. 3-6). On day 14, the TN concentration in R2 (Che. 60) decreased to around 2 mg L^{-1}, however, they were still higher than 3.7 mg L^{-1} in R5 (HTAC 250) and R6 (HTAC 500). On day 35, only the TN concentrations in R5 (HTAC 250) and R6 (HTAC 500) were higher than 1 mg L^{-1}. The TP concentration variation trend was similar with that of TN. Thus, before the summer drainage, the N and P concentrations in the surface water in R5 (HTAC 250), R6 (HTAC 500) exceeded the standard values. If the surface water leaked out, it would cause environmental burden. In the aspect of environment friendly agriculture, the amount of HTAC less than 250 kg N ha^{-1} is recommended for rice growth.

Compared with the chemical fertilizer treatment, the rice yields were higher when the amount of HTAC > 180 kg N ha^{-1}. However, when the amount of HTAC higher than 250 kg N ha^{-1}, the N and P concentrations in the surface water exceeded the standard values just before the summer drainage. Thus the amount of HTAC should be controlled within 250 kg N ha^{-1}, which also would have little risk of rice lodging. Thus, 180 kg N ha^{-1} (R4) was determined as the optimal fertilization amount of HTAC for rice growth.

(2) Variations of N and P in soil

The variation of NH_4^+-N and PO_4^{3-}-P concentrations in soil are shown in Fig. 3-7. The NH_4^+-N concentrations in R1 (NONE), R2 (Che. 60), and R3 (HTAC 60) were lower than 50 mg kg^{-1} from the initial to the final. While in R4 (HTAC 180), R5 (HTAC 250), and R6 (HTAC 500), the initial NH_4^+-N concentrations were 69.7, 73.1 and 108.9 mg kg^{-1}, respectively. The NH_4^+-N concentrations in R5 and R6 firstly decreased, and then increased to the highest (97.5 mg kg^{-1} for R5, 125.9 mg kg^{-1} for R6) on day 28. The PO_4^{3-}-P concentrations increased with increasing fertilization amount in R3-R6. In R1-R3, the PO_4^{3-}-P concentrations varied from 100 mg kg^{-1} to 200 mg kg^{-1}, which were relatively stable. Nevertheless, the PO_4^{3-}-P concentration in R6 was the highest (434.6 mg kg^{-1}) at the initial and then varied around 400 mg kg^{-1}. It decreased to 300 mg kg^{-1} at harvest. While in R5, it varied around 300 mg kg^{-1} and decreased to 200 mg kg^{-1} at harvest.

3.4 Summary

This research first reported the effect of HTAC on rice growth and the surrounding environment. HTAC can enhance the rice yield significantly.

Considering plant quality, rice yield and environmental friendliness, 180 kg N ha^{-1} (R4), which showed similar rice yield with the chemical fertilizer applied, was determined as the optimal fertilization amount of HTAC for rice growth.

The N and P concentrations in the surface water at this fertilization amount can meet the environmental quality standards before being discharged.

Table 3-1 The fertilization conditions for rice growth experiment in 2016.

Treatment	Conditions
R1 NONE	Without fertilizer
R2 Che.60	With 60 kg N ha^{-1} of chemical fertilizer
R3 HTAC 60	With 60 kg N ha^{-1} of HTAC
R4 HTAC 180	With 180 kg N ha^{-1} of HTAC
R5 HTAC 250	With 250 kg N ha^{-1} of HTAC
R6 HTAC 500	With 500 kg N ha^{-1} of HTAC

Table 3-2 The average plant height in panicle formation stage and maturity stage of Koshihikari.

	Average height (cm)	
	Panicle formation stage	Maturity stage
R1 NONE	59.1±3.2 ^{a}	89.7±3.5 ^{a}
R2 Che.60	66.9±2.4 ^{b}	96.8±2.7 ^{b}
R3 HTAC 60	58.9±1.6 ^{a}	92.3±2.0 ^{a}
R4 HTAC 180	68.1±2.6 ^{b}	98.6±2.9 ^{b}
R5 HTAC 250	69.8±3.3 ^{b}	99.1±3.1 ^{b}
R6 HTAC 500	79.6±4.2 ^{c}	107.1±4.5 ^{c}

Data were statistically analyzed by one-way ANOVA with repeated measures and different small letters indicate significant differences ($p < 0.05$) among four treatments of each season by Duncan's method for multiple comparisons. All the data are the means of 8 replicates±standard error.

Table 3-3 Rice yield and yield components.

Conditions	Tillers plant^{-1}	Spikelets plant^{-1}	Ripening rate of grain (%)	Brown rice Weight (g plant^{-1})	1000-grain weight (g)	Rice yield (t ha^{-1})
R1 NONE	11±0.6^{a}	909±50^{a}	77.9 ±1.7^{a}	14.64±2.2^{a}	20.7±0.2^{a}	2.93±0.4^{a}
R2 Che.60	20±1.0^{b}	1463±63^{b}	80.2±1.9^{a}	25.09±3.1^{b}	21.4±0.3^{b}	5.02±0.6^{b}
R3 HTAC 60	11±1.1^{a}	931±49^{a}	82.3±1.6^{b}	15.87±2.5^{a}	20.7±0.1^{a}	3.17± 0.5^{a}
R4 HTAC 180	17±1.5^{b}	1457±74^{b}	84.7±1.4^{c}	26.92±2.8^{b}	21.8±0.2^{b}	5.38±0.6^{b}
R5 HTAC 250	20±1.2^{b}	1618±82^{b}	84.2±1.3^{c}	28.81±3.2^{b}	21.1±0.2^{a}	5.76±0.6^{b}
R6 HTAC 500	29±1.5^{c}	1894±96^{c}	83.0±1.6^{b}	32.81±3.5^{c}	20.9 ±0.3^{a}	6.56±0.7^{c}

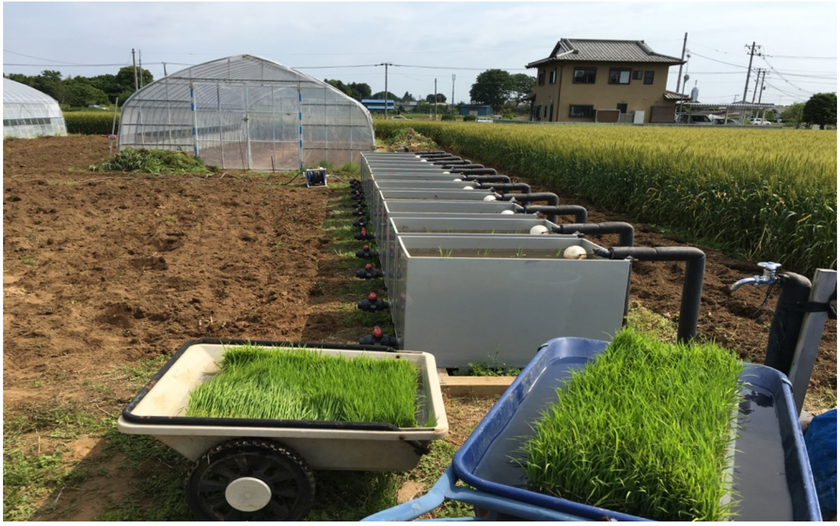
Data were statistically analyzed by one-way ANOVA with repeated measures and different small letters indicate significant differences ($p < 0.05$) among four treatments of each season by Duncan's method for multiple comparisons. All the data are the means of 8 replicates±standard error.

Table 3-4 Summary of the effects of composts on rice yeilds (selected data).

Material	Compost amount	Rice yield(t ha^{-1})	Nations and Time	References
Hyper-thermophilic aerobic compost (HTAC)	None fertilizer	2.93[a]	Japan, May to Septermber, 2016	In this study
	60 kg N ha^{-1} of chemical fertilizer	5.02[b]		
	60 kg N ha^{-1} of HTAC	3.17[a]		
	180 kg N ha^{-1} of HTAC	5.38[b]		
	250 kg N ha^{-1} of HTAC	5.76[b]		
	500 kg N ha^{-1} of HTAC	6.56[c]		
Rice and wheat residues	control	2.41	Pakistan	Sarwar et al., 2007
	Chemical fertilizer	2.99		
	Compost 12t ha^{-1}	2.82		
	Compost 24t ha^{-1}	3.08		
	Compost 24t ha^{-1}+ fertilizer	3.51		
	Compost 24t ha^{-1}+ fertilizer	3.94		
Municipal solid waste	Inorganic fertilizer	5.02	India	Kavitha and Subramanian, 2007
	Municipal solid waste compost	3.78		
	Enriched MSWC	4.13		
	75%N Inorganic + 25%N MSWC	4.69		
	75%N Inorganic + 25%N EMSWC	5.22		
	5t EMSWC + 25%N Inorganic	4.33		
	5t Vermicompost + 25% N Inorganic	4.24		
Cattle dung + maize stover (CDMC)	Control	1.13	Nigeria, June to November, 2011	Dada et al., 2014
	CDMC 8 t ha^{-1}	5.17		
Poultry dropping + maize stover (PDMC)	PDMC 8 t ha^{-1}	4.91		
--	No fertilizer	4.51	Bangladesh, July to November, 2013	Hossen et al., 2015
	Inorganic fertilizer	4.52		
	Compost 10 t ha^{-1}	5.2		
	Compost 20 t ha^{-1}	5.8		
	Compost 30 t ha^{-1}	5.13		
	Compost 50 t ha^{-1}	5.1		

Rice straw	Fertilizer 0	2.36[c]	Vietnam, 2012 wet season	Watanabe et al., 2017
	Fertilizer 0 + Compost 6000 kg ha^{-1}	3.63[b]		
	Fertilizer 40 kg ha^{-1}	3.68[b]		
	Fertilizer 40 kg ha^{-1} + Compost 6000 kg ha^{-1}	4.60[a]		
	Fertilizer 60 kg ha^{-1}	3.70[b]		
	Fertilizer 60 kg ha^{-1} + Compost 6000 kg ha^{-1}	4.74[a]		
	Fertilizer 100 kg ha^{-1}	3.72[b]		
Crushed cotton gin	non-fertilized	0.9023[a]	Spain, April to October, 2000	Tejada and Gonzalez, 2006
	Crushed cotton gin compost (CC), 10 t ha^{-1}	0.9609[b]		
	CC 15 t ha^{-1}	0.9698[b]		
	CC 20 t ha^{-1}	0.9784[b]		
	250 kg N ha^{-1} + CC 10 t ha^{-1}	0.9766[b]		
	250 kg N ha^{-1} + CC 15 t ha^{-1}	0.9851[b]		
	250 kg N ha^{-1} + CC 20 t ha^{-1}	0.9946[bc]		
Sewage sludge	non-fertilized	1.44[d]	India, between July and November , 2005	Singh and Agrawal, 2010
	150 kg N ha^{-1}	2.29[c]		
	160 kg N ha^{-1}	3.03[b]		
	180 kg N ha^{-1}	3.24[ab]		
	190 kg N ha^{-1}	3.36[a]		
	210 kg N ha^{-1}	3.40[a]		

(a)



(b)

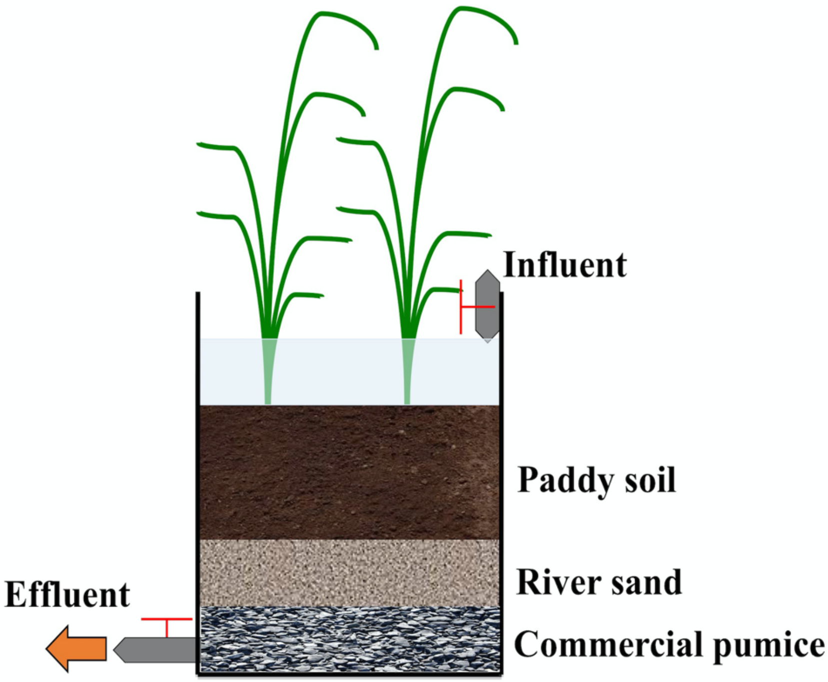
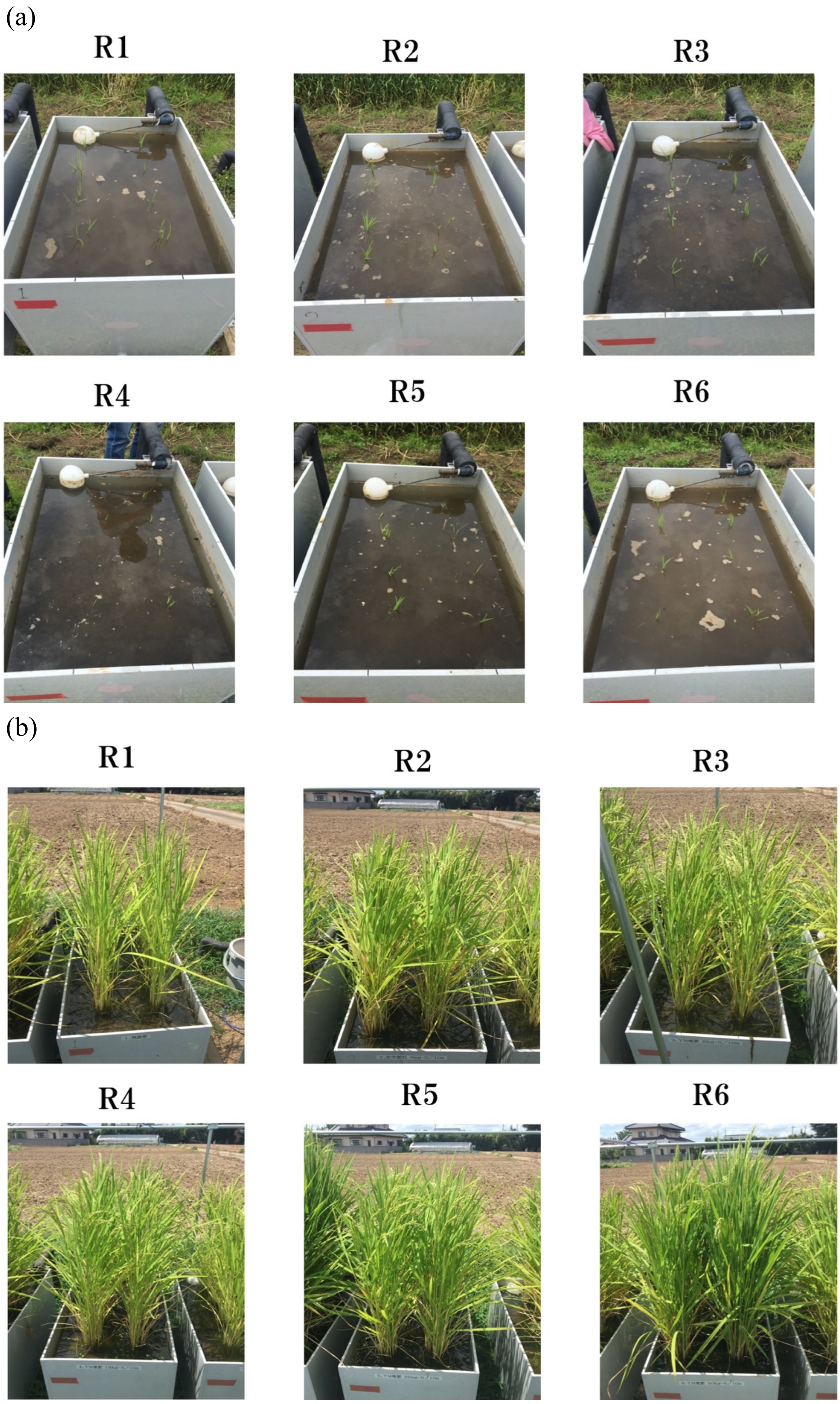


Fig. 3-1 Images of study site (a) and experimental device (b).


(a)
R1
R2
R3
R4
R5
R6
(b)
R1
R2
R3
R4
R5
R6

(c)

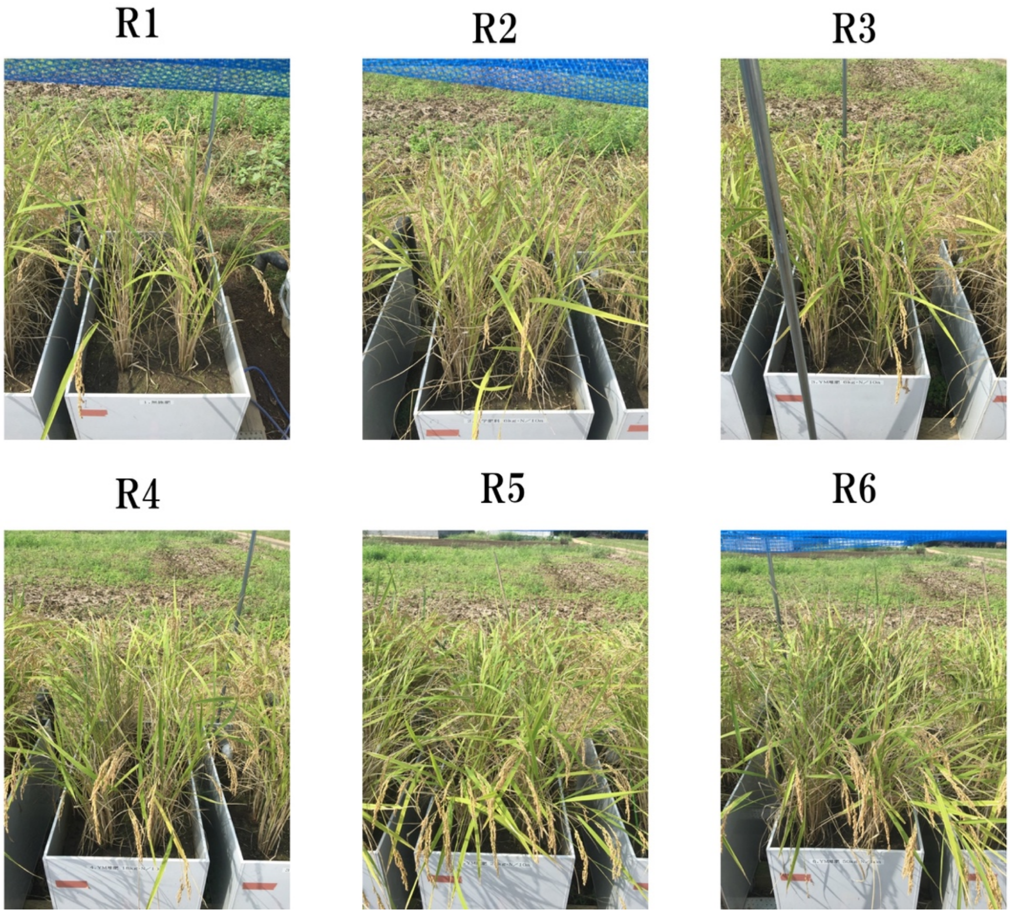


Fig. 3-2 The image of rice on day 1(a, planting stage), day 84 (b, panicle formation stage) and day 115 (c, maturity stage).

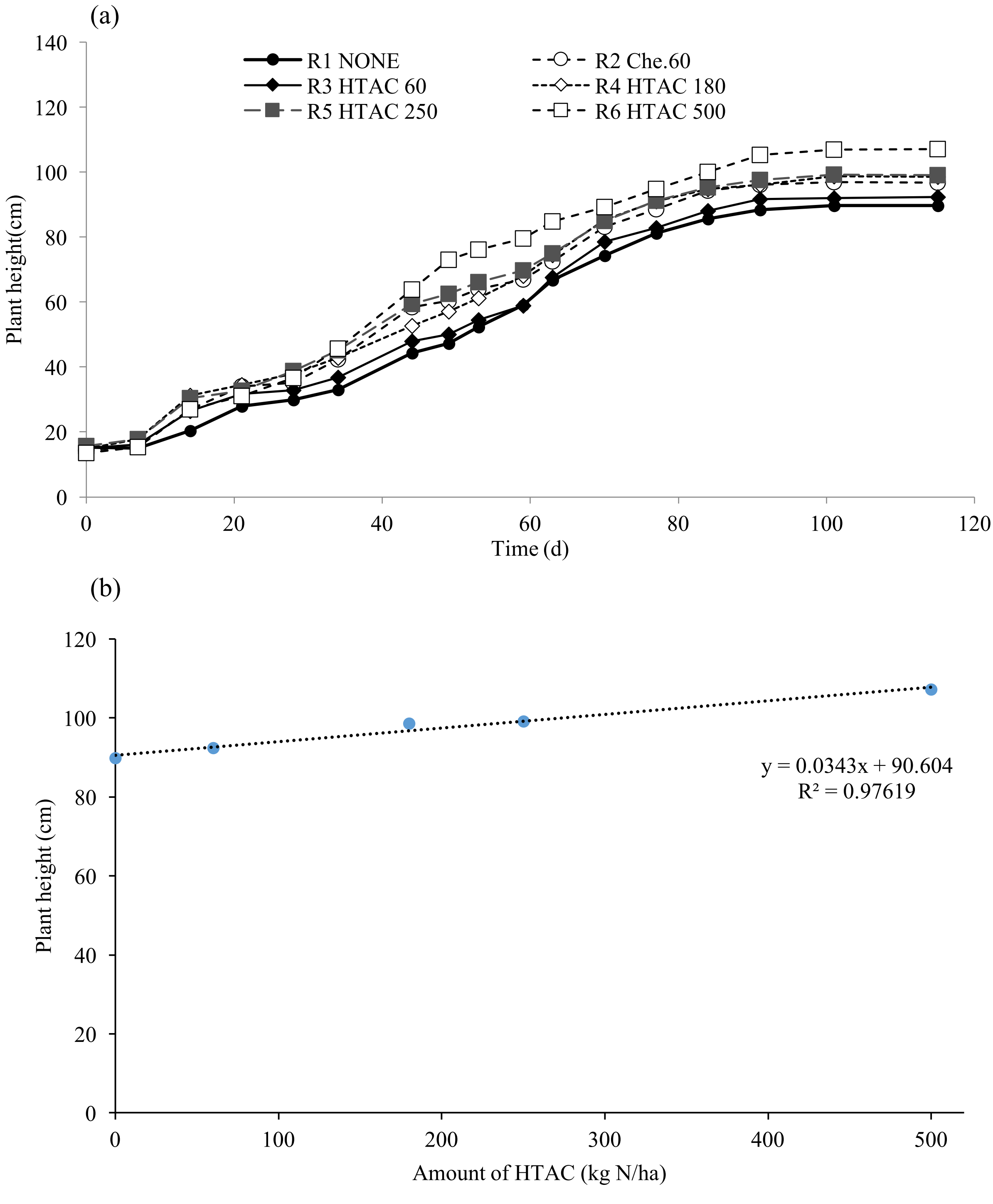


Fig. 3-3 Variation of plant height in R1-R6 with different treatment conditions (a) and the relationship between HTAC amount and plant height on day 115 (b).

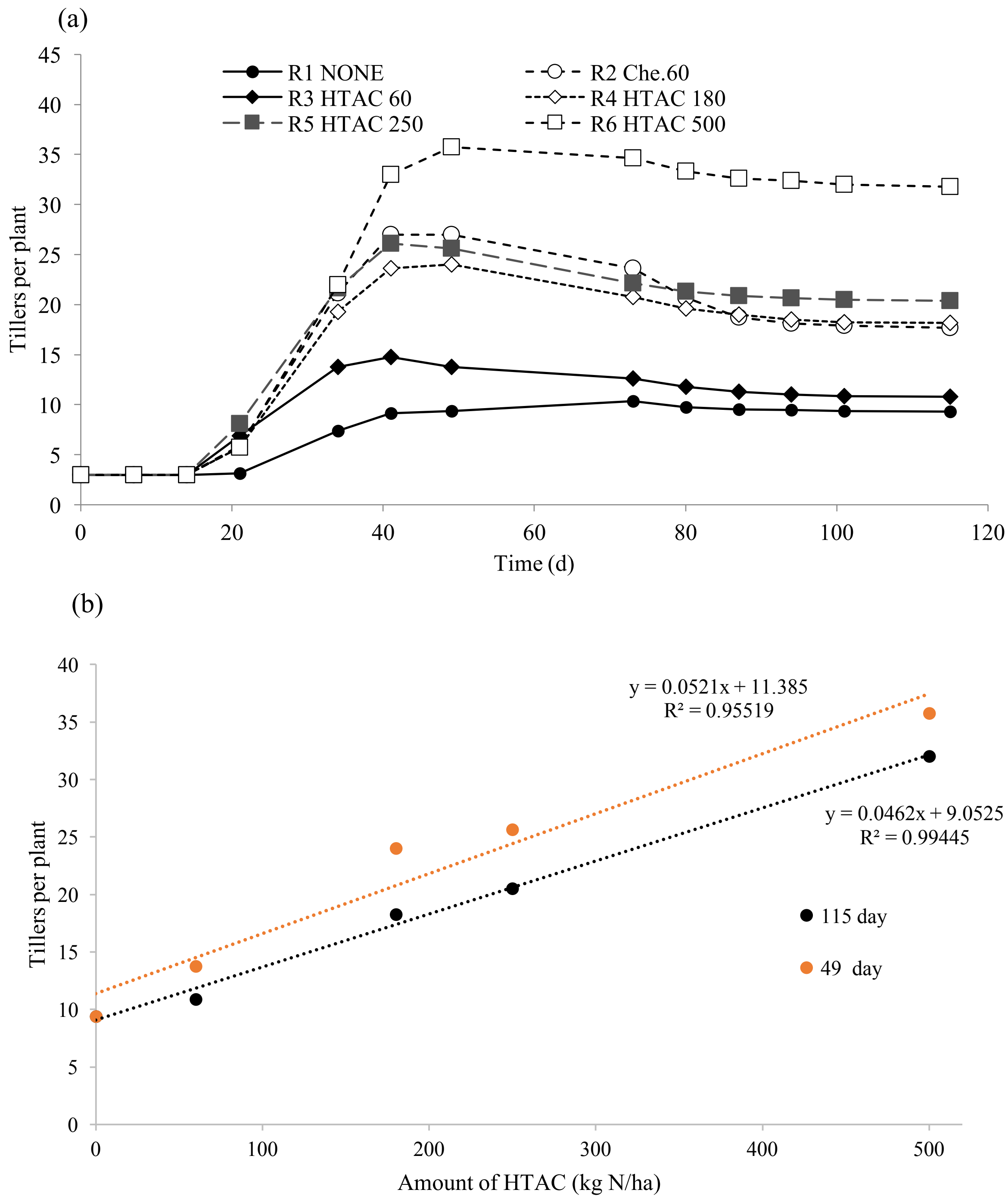


Fig. 3-4 Variation of tillers per plant in R1-R6 with different treatment conditions (a) and the relationship between HTAC amount and tillers number on day 49 and day 115 (b).

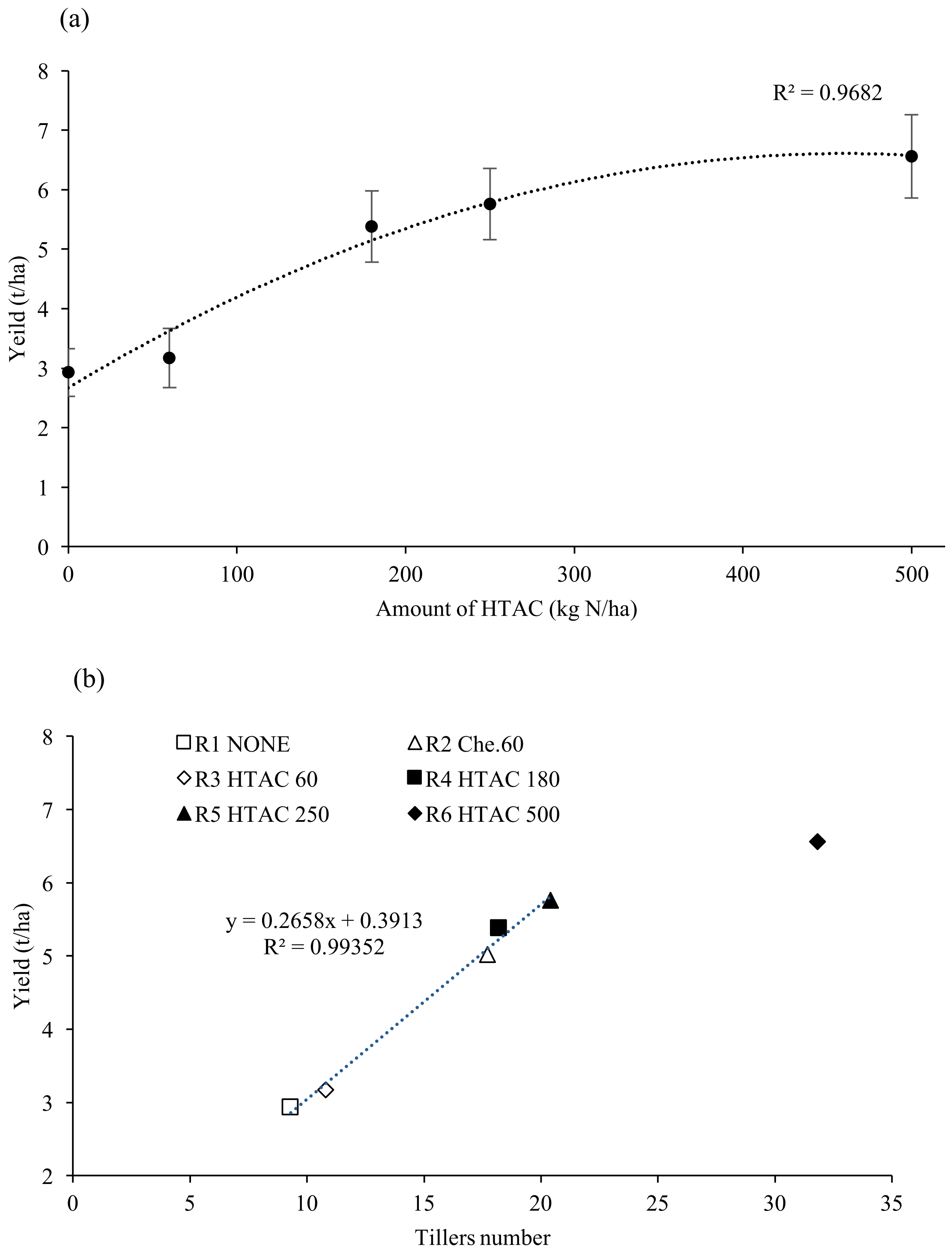


Fig. 3-5 The relationship between rice yield and HTAC amount (a) and tillers number (b).

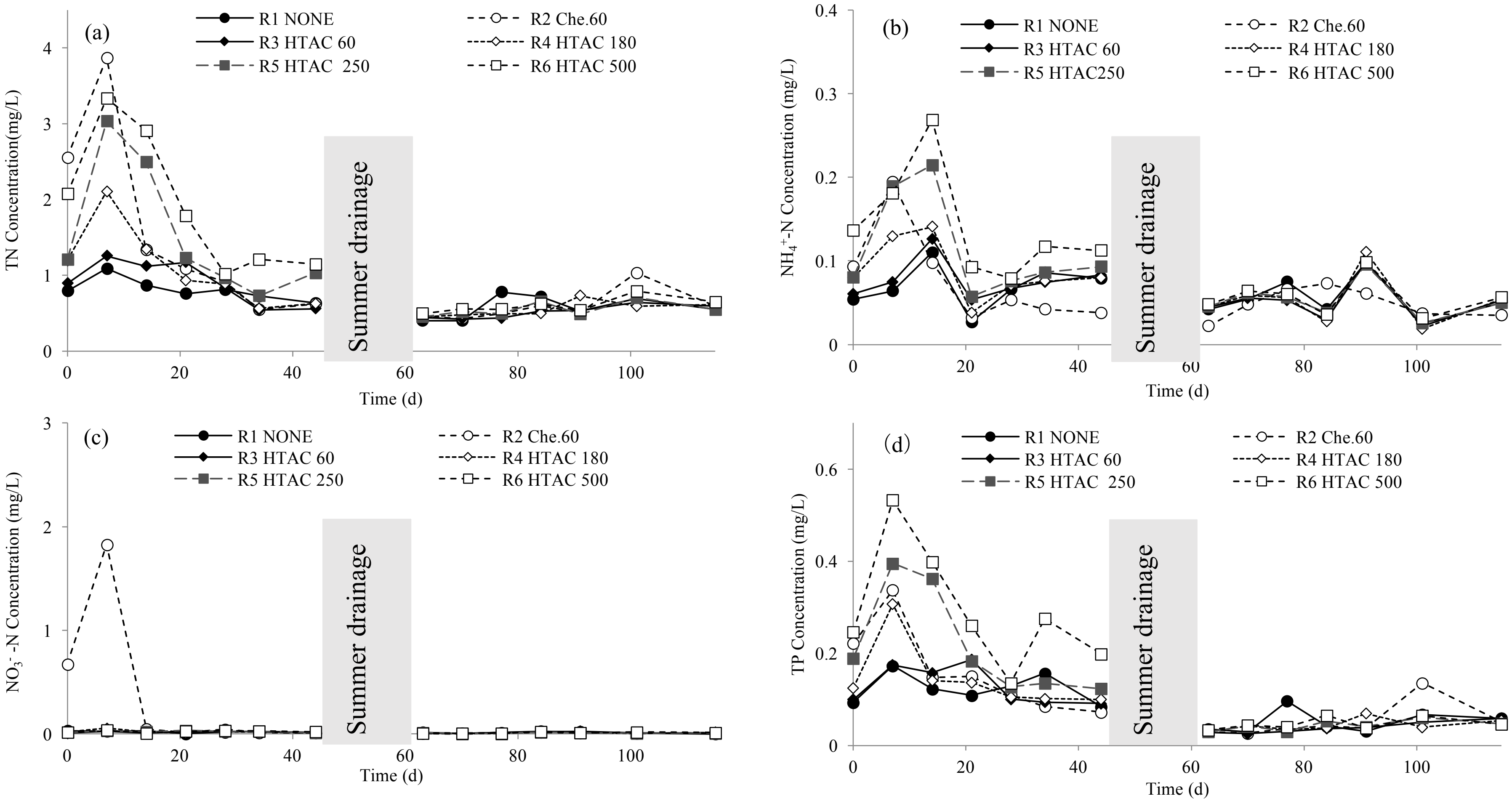


Fig. 3-6 Variations of TN, NH_4^+-N, NO_3^--N, TP concentrations in surface water under different treatment conditions. a, TN concentration; b, NH_4^+-N concentration; c, NO_3^--N concentration; d, TP concentration.

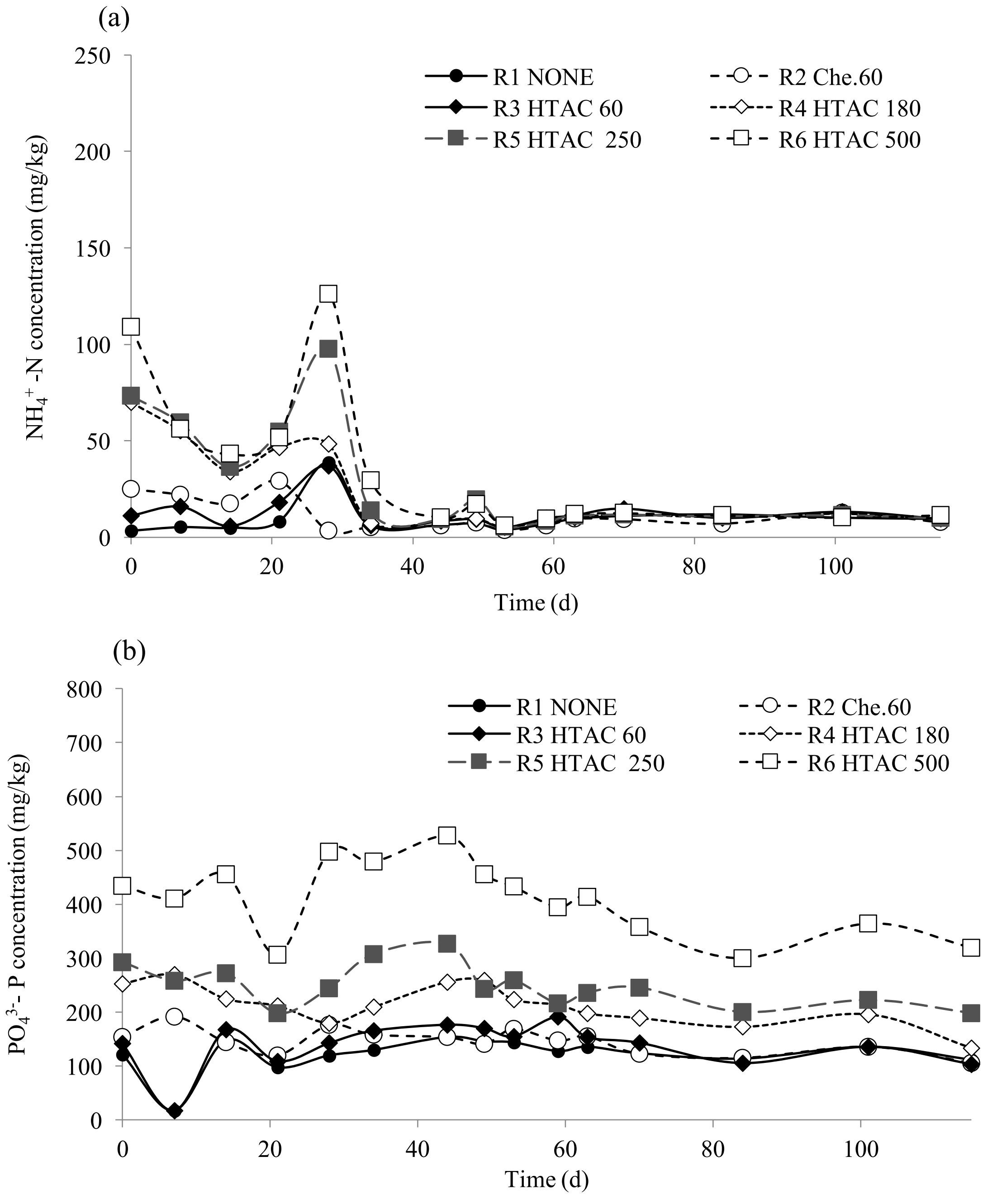


Fig. 3-7 Variations of NH_4^+-N (a) and PO_4^{3-}-P (b) concentrations in soil under different treatment conditions.

Chapter 4 Comparison between hyper-thermophilic aerobic compost and submerged plants for rice growth and yield

4.1 Introduction

Submerged macrophytes are an important component for the aquatic primary production and biogeochemical cycles. Submerged macrophytes can reduce water turbidity caused by phytoplankton and suspended solids, which exhibit better nitrogen removal efficiency than emergent macrophytes. However, when submerged macrophytes are excessively grown, serious environmental problems would occur in lakes, dams and reservoirs worldwide (Vincent, 2001, Marcus et al., 2003, Zhang et al., 2011). For instance, the serious invasion of the submerged macrophyte *Egeria densa*, which is originated from North America, is occurred in many European countries (Escobar et al., 2011). The Lake Biwa, the largest lake in Japan (674 km^2), has been excessively propagated by submerged macrophytes, covering approximately 90% of the southern basin since 1994 (Haga and Ishikawa, 2011). The large quantity of phytomass has been causing water stagnation, foul odor, fishing interference, ecosystem change and landscape fouling (Haga et al., 2006). Every year, more than 2600 tons (wet weight) of the submerged macrophytes are removed from the lake and the harvesting cost reaches greater than USD 2.0 million per annum (Kawanabe et al., 2012).

Effective and low-cost treatments are necessary for treating the excessively submerged macrophytes. Although anaerobic digestion is one of the effective technology to recover energy from the harvested submerged macrophytes (Abbasi et al., 1990; O'Sullivan et al., 2010), the equipments required for anaerobic digestion are also costly. On the other hand, only around half of the organics can be anaerobically digested, and the rich nutrients inside submerged macrophytes still need to be recovered afterwards.

Another method is to use submerged macrophytes as green manure. Green manures play important roles in supplying nutrients for crops, improving ecological environments of agricultural fields, reducing soil erosion and pollution (Becker et al., 1995), restraining global warming potentials (Robertson, 2000), and contributing to higher crop yields (Tejada et al. 2008). Green manures can be fitted into rice farming systems in either the pre-rice or post rice phase (Garrity and Flinn, 1988). Many studies reported that the application of green manures changed the chemical and biological characteristics of soils (Elfstrand et al., 2007; Bernard et al., 2012). Milk vetch, one kind of winter-growing legumes, has been widely used as green manure in rice fields to fertilize the soils in Japan and China (Samarajeewa et al., 2005). However, little information could be found on reusing submerged macrophytes as green manure.

Submerged plants can absorb abundant nutrients such as nitrogen and phosphorus from the watershed thus containing abundant nitrogen and phosphorus. If the submerged plants could be successfully used as organic fertilizer, the eutrophication in lakes can be more effectively solved with environmental friendliness. *Egeria densa* is a rooted multi-branched perennial plant but can survive and grow as floating fragments. The dark green blade-like leaves (3/5-inch-long and 1/5-inch-wide) are in whorls of three with finely toothed margins. An aquatic plant, *C. demersum* has stems that reach lengths of 1-3 m (3-10 ft), with numerous side shoots making a single specimen appear as a large, bushy mass. The leaves are produced in whorls of six to twelve, each leaf was 8-40 mm long, simple, or forked into two to eight thread-like segments edged with spiny teeth; they are stiff and brittle. It is monoecious, with separate male and female flowers produced on the same plant. The flowers are small, 2 mm long, with eight or more greenish-brown petals; they are produced in the leaf axils. Coontail (*C. demersum*) is a perennial and widely distributed submerged macrophyte. The stems, leaves and epidermis of submerged macrophytes can assimilate nutrients (Dai et al., 2012, Paterniti and Mantai, 1986). Nevertheless, coontail decomposes easily after death. Chimney and Pietro (2006) reported that the decomposition rate (0.056 d^{-1}) of coontail was higher than other macrophytes, indicating its high fertilizer potential.

In this research, HTAC was further applied for the second year in order to elucidate the long-term application effect. In addition, the fertilizer potential and applicability of the mixture of HTAC and submerged plants *C. demersum* and *Egeria densa* with the same nitrogen content of 180 kg N ha^{-1} (optimum dosage obtained in Chapter 3) at different mixing ratios were analyzed.

4.2 Materials and methods

4.2.1 Fertilizer

(1) HTAC

HTAC used in this research was the same as in Chapter 2 and Chapter 3.

(2) Submerged plants

The plants (Fig. 4-1) were sampled from Water Environment Conservation Revitalization Research Station in Japan. The characteristics of submerged plants are shown in Table 4-1.

Before using as fertilizer, the plants was crumbled and mixed with soil by shovel.

4.2.2 Study site and rice growth experiment

The rice growth experiment was performed on a farmland at Tsukuba, Ibaraki, Japan (36°05′N,140°04′) in 2017 (Fig. 4-2). All the other conditions were the same as Chapter 3.2.2. The rice growth periods were from May 23, 2017 to September 27, 2017. The fertilization conditions are shown in Table 4-2. The soil used in 2016 were continuously used in this year in R1-R5. The soil for R7-R12 were thoroughly mixed to reduce experimental error. Midsummer drainage was conducted between day 44 and 63 during rice growth experiment.

The calculations of rice yield and yield components, and statistic analysis were the same as Chapter 3.2.3 and 3.2.5, respectively.

4.3 Results and discussion

4.3.1 Characteristics of rice growth

(1) Plant height

The variation of plant height over time are shown in Fig. 4-3. In R3-R5 with the increase in the fertilization amount of HTAC, the plant height was found to be positively correlated with the fertilization amount. The highest plant height was reached to 107.9 cm in R2 (Che. 60) after 101 days' growth.

The stem height, tillers number and rice yields in R6-R12 significantly increased compared with the control (none fertilizer). Compared with R2 (chemical fertilizer), rice yields in R6-R12 were lower.

In this research, the rice growth was normal after transplanting in all the treatment conditions. No matter the second year HTAC utilization or the submerged plant application, no death or growth defect happened in all the treatments. Seen from Fig. 4-3b, the plant height in R6-R12 was higher than the control, but there was no significant difference between HTAC and submerged plants treatment.

(2) Rice tillers per plant

The variation of tillers per plant are shown in Fig. 4-4. After transplanting, the number of stem increased with the occurrence of tiller and reached the maximum value. Then the ineffective tillers died, and panicle growth from remained tillers. All treatments reached the maximum tiller number on day 49. Tiller numbers in R6-R12 were 11.8, 35.8, 11.0, 17.8, 19.3, 16.3 in R1 to R6, respectively. Among all the treatments, R2 (che. 60) achieved the highest tiller number.

The numbers of panicles from R10 to R12 at harvest were 14.75, 15.00, and 15.25, respectively. It seems that the application of *C. demersum* as organic fertilizer had the same effect as HTAC. On the other hand, the numbers of panicles from R7 to R9 applying *Egeria densa* and HTAC were 14.75, 13.00, 14.50, respectively. In R8, especially a low value were noted, while during the same period the plant height was higher than R7 and R9. This suggested that probably the long-range phenomenon occurred. When applying *Egeria densa* together with HTAC as organic fertilizer, it is necessary to optimize the mixing ratio of them.

(3) The panicle number

The first stage of rice growth is panicle appearance. In 2017, the panicle in all the treatments did not appear at the same time. The panicle was first occurred in R11 on day 68. Table 4-3 shows the panicle number and the percentage of panicle number to tillers number on day 73. The highest panicle number was obtained in R11, more than 10 on day 73. From R1 to R5, the percentage was all lower than 50%; however, in R6-R12, the percentage were all higher than 50% except for R7 and R10. In R2, only 6.8% tillers appeared panicles. This may be due to in R2, the tillers number and plant height were significantly higher than others. Nutrients may first support the growth of tillers. From Table 4-4, the ripening rate of grain in R2 was also the lowest, indicating that the panicle appearance time may have influences on the results of ripening rate of grain.

4.3.2 Yield and yield components

As shown in Table 4-4, the rice yields in R3-R5 with only HTAC significantly increased with the increase in fertilization amount. The highest rice yield was 4.79 t ha^{-1} in R2 (Che. 60). In R3-R5, the rice tiller number and spikelet number per plant also increased with the increase in fertilization amount, while the ripening rate of grain and 1000-grain weight decreased. They all showed similar trends compared with those in 2016.

The rice yields in R2, R3 and R4 were 1.83, 1.07 and 1.74 times higher than that of the control, respectively. Higher increase of rice yield (1.74 times) was achieved in this study by using only HTAC of 180 kg N ha^{-1}, indicating the good quality of HTAC for rice growth.

The rice yields in R6 to R12 were 1.51, 1.38, 1.23, 1.48, 1.29, 1.28, and 1.34 times higher than that of the control, respectively. Compared to the HTAC, the submerged plants also enhanced the yield of rice.

Table 4-5 summarizes the effects of green manure on rice yields. Yao et al. (2017) studied the duckweed as green manure for rice growth, urea combined with duckweed achieving higher rice yield by 9–10% over the control; however, using the conventional rate of 300 kg N ha^{-1}did not increase rice yield over using the reduced N rate of 225 kg N ha^{-1}, with or without duckweed. Xie et al. (2016) studied fertilizer and Legumes for rice growth. Their results show that green manure substitution for fertilizer at appropriate rates (e.g., 20–40%) has the potential to maintain sustainable production for rice grain yield in south China. Kim et al. (2012) used rye and milk vetch as green manure with fertilizer for rice growth. Results indicated that plant growth and yield components were significantly improved with chemical fertilization and green manure. NPK fertilization significantly increased grain yield to 34–46% over the control. Leguminous milk vetch application was more effective on improving rice growth and yield properties than non-leguminous rye.

The rice yields in the submerged plant treatments were higher than control, but lower than the fertilizer treatment. Compared with other research, green manure with fertilizer was higher than the fertilizer alone at same nitrogen content. So in the future the effect of submerged plant and fertilizer on rice yield needs to be investigated.

4.4 Summary

In the second-year application, 180 kg N ha^{-1} (R4) as the optimal fertilization amount of HTAC for rice growth obtained from the first year was re-examined alone and together with the submerged plants. No inhibition was noticed in the two-year application.

Submerged plants *C. demersum* and *Egeria densa* were also found to enhance the rice yield significantly, which can be used as organic fertilizer. These two plants have the same effects as HTAC at the same nitrogen level.

Table 4-1 Characteristics of submerged planted in this study.

	Egeria densa	*C. demersume*
TS (%)	6.6	5.1
TC (%)	35.1	37.1
TN (%)	2.9	5.2
TP (%)	0.86	1.7
C/N ratio	12.1	7.1
TK (%)	4.1	4.8
CaO (%)	2.5	1.7
Mg (%)	0.43	0.82
Fe (%)	0.05	0..03
Zn (%)	0.07	0.10
Mn (%)	0.13	0.06

*TS, total solids content; TC, total carbon; TN, total nitrogen content; TP, total phosphorus content; TK, total potassium.

**The data were measured by Ibaraki pharmaceutical association examination center Co., Ltd.

Table 4-2 The fertilization conditions for rice growth experiment in 2017.

Treatment	Conditions
R1 NONE	Without fertilizer
R2 Che.60	With 60 kg N ha^{-1} of chemical fertilizer
R3 HTAC 60	With 60 kg N ha^{-1} of HTAC
R4 HTAC 180	With 180 kg N ha^{-1} of HTAC
R5 HTAC 250	With 250 kg N ha^{-1} of HTAC
R6 HTAC 180	With 180 kg N ha^{-1} of HTAC
R7 El. 180	With 180 kg N ha^{-1} of *Egeria densa*
R8 El. 120	With 120 kg N ha^{-1} of *Egeria densa*, 60 kg N ha^{-1} of HTAC
R9 El. 60	With 60 kg N ha^{-1} of *Egeria densa*, 120 kg N ha^{-1} of HTAC
R10 CD 180	With 180 kg N ha^{-1} of *Ceratophyllum demersum*
R11 CD 120	With 180 kg N ha^{-1} of *Ceratophyllum demersum*, 60 kg N ha^{-1} of HTAC
R12 CD 60	With 60 kg N ha^{-1} of *Ceratophyllum demersum*, 120 kg N ha^{-1} of HTAC

* The soil in R6-R12 was thoroughly mixed before fertilized.

Table 4-3 The panicle number and the percentage of panicle number to tiller number on day 73.

	R1	R2	R3	R4	R5	R6
Panicle number	3.0[ab]	2.2[a]	3.8 [ab]	8.6[cd]	7.5[cd]	9.0[d]
Panicle number /tiller number	26.8%[b]	6.8%[a]	28.9%[b]	50.2%[cde]	42.4%[bcd]	54.4%[de]
	R7	R8	R9	R10	R11	R12
Panicle number	5.8[bc]	7.8[cd]	9.1[d]	4.1[ab]	10.5[d]	10.1[d]
Panicle number /tiller number	35.6%[bc]	56.6%[de]	56.2%[de]	26.5%[b]	61.6%[e]	59.4%[de]

* On day 68, the panicle began to show in R11.

**Data were statistically analyzed by one-way ANOVA with repeated measures and different small letters indicate significant differences (p<0.05) among four treatments of each season by Duncan's method for multiple comparisons. All the data are the means of 8 replicates ± standard error.

Table 4-4 Rice yield and yield components in 2016 and 2017.

Conditions	Tillers plant^{-1}	Spikelets plant^{-1}	Ripening rate of grain (%)	Brown rice Weight (g plant^{-1})	1000-grain weight (g)	Rice yield (t ha^{-1})
2016						
R1 NONE	11±0.6^{a}	909±50^{a}	77.9 ±1.7^{a}	14.64±2.2^{a}	20.7±0.2^{a}	2.93±0.4^{a}
R2 Che.60	20±1.0^{b}	1463±63^{b}	80.2±1.9^{a}	25.09±3.1^{b}	21.4±0.3^{b}	5.02±0.6^{b}
R3 HTAC 60	11±1.1^{a}	931±49^{a}	82.3±1.6^{b}	15.87±2.5^{a}	20.7±0.1^{a}	3.17± 0.5^{a}
R4 HTAC 180	17±1.5^{b}	1457±74^{b}	84.7±1.4^{c}	26.92±2.8^{b}	21.8±0.2^{b}	5.38±0.6^{b}
R5 HTAC 250	20±1.2^{b}	1618±82^{b}	84.2±1.3^{c}	28.81±3.2^{b}	21.1±0.2^{a}	5.76±0.6^{b}
R6 HTAC 500	29±1.5^{c}	1894±96^{c}	83.0±1.6^{b}	32.81±3.5^{c}	20.9 ±0.3^{a}	6.56±0.7^{c}
2017						
R1 NONE	10±0.9^{a}	715±28^{a}	88.9±2.6^{d}	13.05±0.3^{a}	20.5±2.0^{a}	2.61±0.2a
R2 Che.60	26±1.5^{c}	1427±45^{d}	78.4±1.7^{a}	23.93±1.2^{c}	21.4±1.5^{b}	4.79±0.4^{c}
R3 HTAC 60	11±1.2^{a}	706±39^{a}	88.4±7.3^{d}	13.92±0.7^{a}	22.1±0.2^{b}	2.79±0.1^{a}
R4 HTAC 180	16±1.0^{b}	1153±23^{c}	85.8±3.8^{c}	22.61±0.9^{c}	22.8±0.6^{c}	4.53±0.2^{c}
R5 HTAC 250	16±1.2^{b}	1251±98^{c}	86.5±7.3^{c}	23.55±0.2^{c}	21.8±0.5^{b}	4.71±0.4^{c}
R6 HTAC 180	14±0.9^{b}	1017±32^{c}	89.5±5.7^{d}	19.64±1.0^{b}	21.6±1.0^{b}	3.93±0.3^{b}
R7 El. 180	15±1.2^{b}	1018±66^{c}	86.6±2.2^{c}	17.98±1.4^{b}	20.4±0.4^{a}	3.60±0.3^{b}
R8 El. 120	13±1.1^{b}	908±78^{b}	83.5±5.5^{b}	16.10±0.4^{b}	21.5±1.1^{b}	3.22±0.3^{b}
R9 El. 60	14±0.8^{b}	995±83^{b}	87.1±6.0^{c}	19.31±0.3^{b}	22.2±0.3^{b}	3.86±0.3^{b}
R10 CD 180	15±1.4^{b}	939±57^{b}	82.7±2.2^{b}	16.82±0.6^{b}	21.7±2.1^{b}	3.37±0.2^{b}
R11 CD 120	16±0.8^{b}	885±60^{b}	83.2±6.4^{b}	16.62±1.0^{b}	22.6±1.1^{c}	3.33±0.1^{b}
R12 CD 60	15±0.3^{b}	888±34^{b}	86.3±6.7^{c}	17.50±0.2^{b}	22.8±2.1^{c}	3.50±0.1^{b}

*Data were statistically analyzed by one-way ANOVA with repeated measures and different small letters indicate significant differences ($P<0.05$) among four treatments of each season by Duncan's method for multiple comparisons. All the data are the means of 8 replicates ± standard error.

Table 4-5 Summary of the effects of green manure on rice yeilds (selected data).

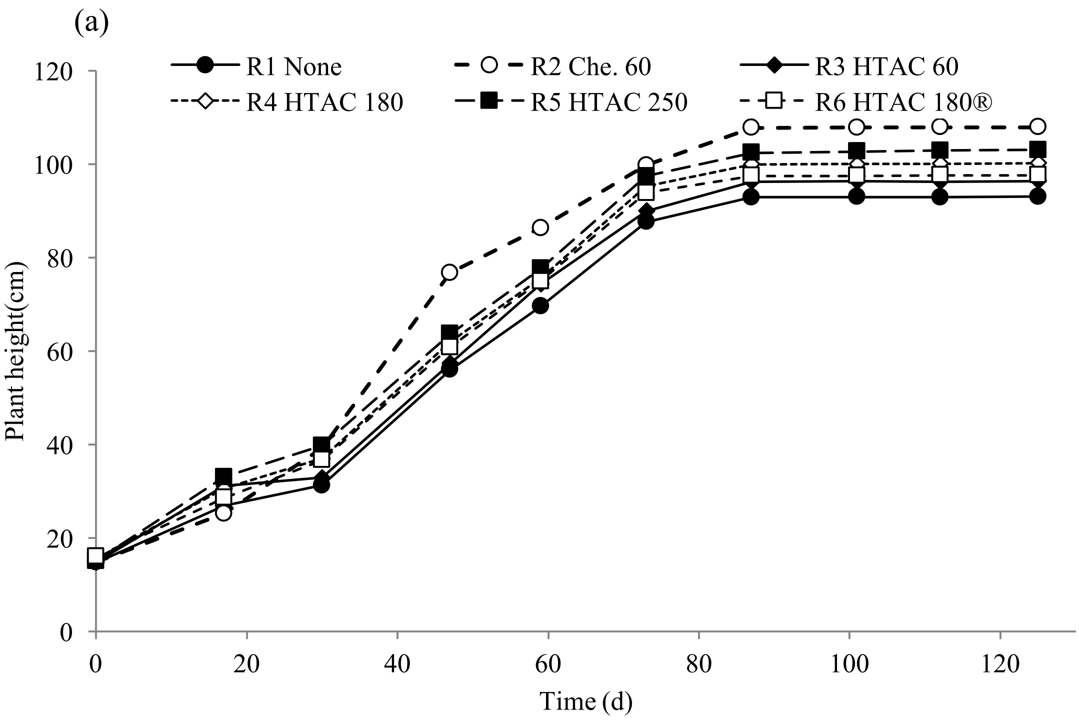
Green munure	amount	Rice yield(t ha^{-1})	Nations and Time	References
Duckweed	no N-fertilize	4.88	China, 2014	Yao et al., 2017
	urea 225 kg N ha^{-1}	7.80		
	urea with duckweed at 225 kg N ha^{-1}	8.66		
	urea 300 kg N ha^{-1}	7.68		
	urea with duckweed at 300 kg N ha^{-1}	8.80		
Fertilizer N (FN) Legumes (GM)	no FN	2.78^{d}	China, 2013	Xie et al., 2016
	100% FN 150 kg N ha^{-1}	4.99^{a}		
	80% FN 20% GM	5.33^{a}		
	60% FN 40% GM	4.96^{ab}		
	40% FN 60% GM	4.69^{c}		
	20% FN 80% GM	4.42^{c}		
Rye Milk vetch	no fertilization	3.5^{c}	South Korea, 2009	Kim et al., 2012
	NPK (mineral fertilizer alone)	4.5^{b}		
	NPK + rye	5.1^{b}		
	NPK + milk vetch	6.6^{a}		

(a)

(b)

Fig. 4-1 Images of *Ceratophyllum demersum* (a) and *Egeria densa* (b).

Fig. 4-2 Images of mixing soil, collecting submerged plants and fertilizer submerged plants.



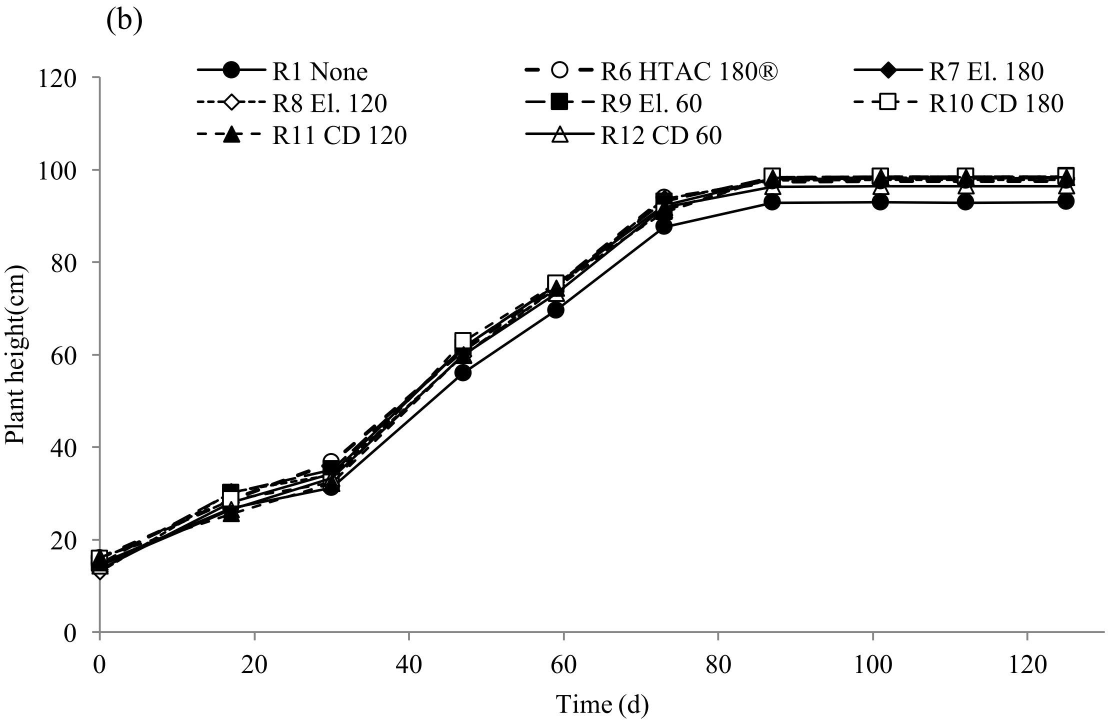
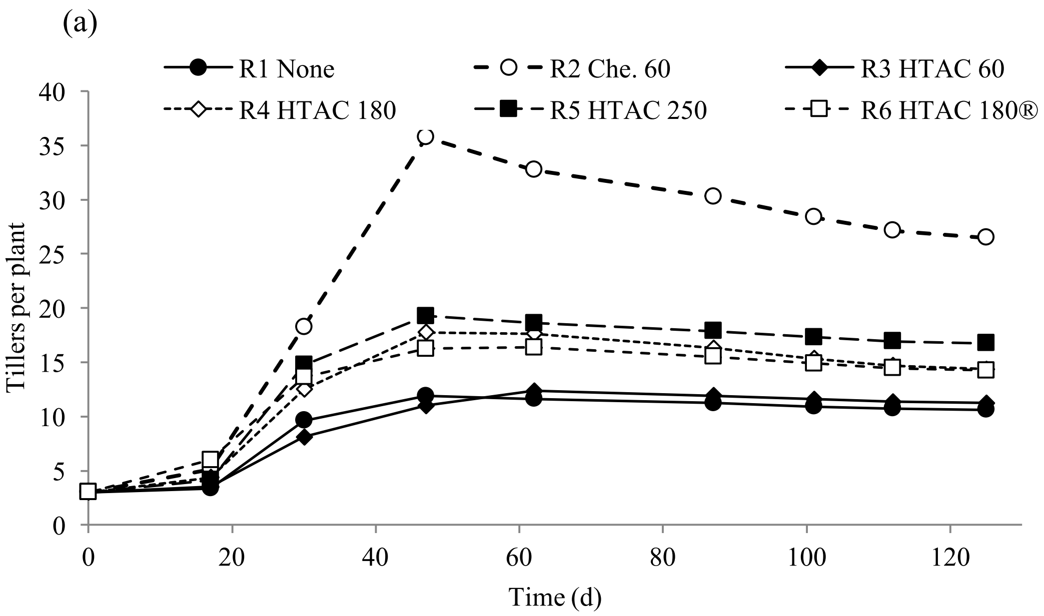


Fig. 4-3 Variations of plant height under different treatment conditions.



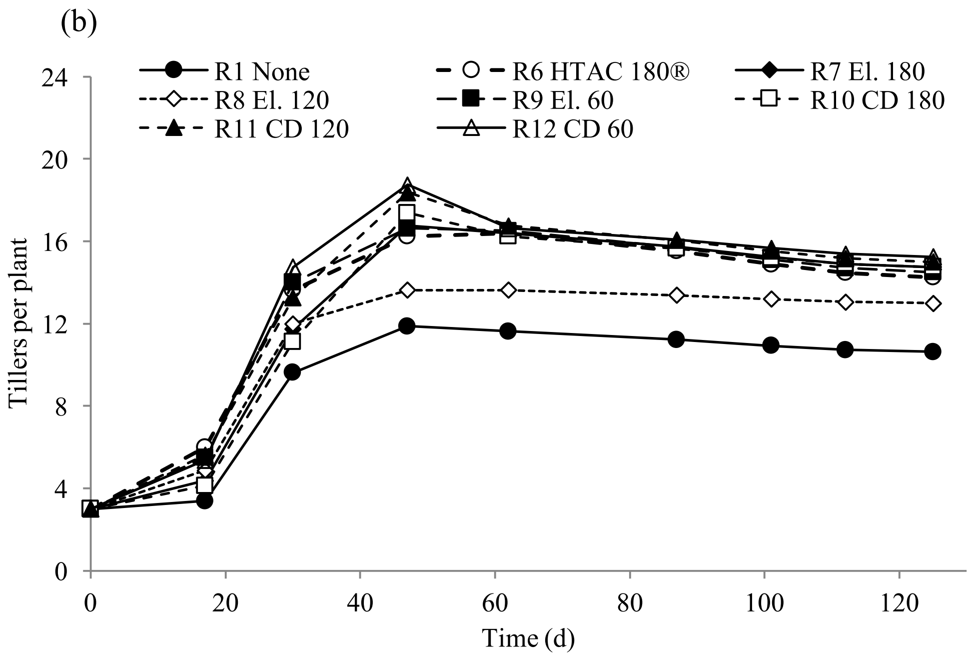


Fig. 4-4 Variations of tillers per plant under different treatment conditions.

Chapter 5 Conclusions and future researches

In this Chapter, the results from Chapter 2, Chapter 3 and Chapter 4 were concisely concluded, and future researches were also prospected.

5.1 Conclusions

Sewage sludge contains large amount of nutrients that can be recovered and used for crop growth. HTAC manufacturing could produce organic fertilizer from sewage sludge during a shorter period. However, there are no research reported the safety and fertilizer quality of the compost produced through this process. HTAC contains not only mineralized nutrients, but also organic nutrients that can be slowly decomposed to release nutrients for plant growth. Thus, it is also difficult to determine the fertilization amount of the compost. Overdosing would induce environmental pollution and long-lived phenomena in rice crops, and infect the rice yield.

(1) The results from seed germitation test showed that HTAC had no phytotoxicity when it was diluted by 20 times. This indicated that the dilution time was also an important factor affecting the phytotoxicity of compost. HTAC not only has no phytotoxicity, but also can improve the elongation of plant stem. However, the lengths of root in all the HTAC treatment conditions were shorter than that in distilled water treatment, which might be resulted from the high ammonium concentration in HTAC. Thus, more attention should be paid on ammonium toxicity when applying HTAC on farmland.

(2) The results from Komatsuna cultivation experiment again indicated that ammonium nitrogen might limit the elongation of radicle. The fresh weight of stem and root in R3 (10.64 g L^{-1} HTAC) was significantly higher than other treatments, thus the optimum dosage of HTAC for soilless cultivation of Komatsuna was 10.64 g L^{-1} in this study.

(3) This research first reported the effect of HTAC on rice growth and the surrounding environment. The results from rice growth experiment in the first year (2016) indicated that HTAC can enhance the rice yield significantly. When considering plant quality, rice yield and environmental friendliness, 180 kg N ha^{-1} (R4) with similar rice yield as in chemical fertilizer test, was determined as the optimal fertilization amount of HTAC for rice growth. The N and P concentrations in the surface water at this fertilization amount can also meet the environmental quality standards before being discharged.

(4) The long-term application effect of HTAC was further investigated by applying it for

the second year's field experiment. The similar results were obtained with that in the first year.

(5) Submerged plants *C. demersum* and *Egeria densa* were also found to enhance the rice yield significantly, which can be used as organic fertilizer. These two plants have the same effects as HTAC at the same nitrogen level.

Therefore, the results obtained from this study provide significantly important reference for nutrients utilization in sewage sludge treated by HTAC.

5.2 Future researches

(1) According to the results of Komatsuna cultivation in this study, the weight of root and stem in R3 (10.64 g L^{-1} HTAC) was the highest. However, compared with the normal yield of Komatsuna, the result obtained in this study is still low. In the future study, the other factors, such as light intensity, temperature and humidity, should be further investigated in the soilless culture system.

(2) In this study, the safety and quality of HTAC was confirmed. Considering plant quality, rice yield and environmental friendliness, 180 kg N ha^{-1} (R4), showing the similar rice yield with that obtained in the chemical fertilizer treatment, was deemed as the optimal fertilization amount of HTAC for rice growth. However, organic fertilizer is not only used by plants, but also used by microorganisms and then improve the the properties of soil. Thus, the effect of HTAC on soil properties needs further investigation.

(3) The rice yield in submerged plant treatments was higher than the control, but still lower than the fertilizer treatment. Compared with other research, green manure with fertilizer was higher than the chemical fertilizer treatment alone at the same nitrogen content. Thus, in the future the effect of submerged plant and fertilizer on rice yield needs further confirmation.

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Appendix

1. Yanfei Cheng, Ryuhei Inamori, Kakeru Ruike, Yuhei Inamori, Zhenya Zhang. Optimum Dosage of Hyper-Thermophilic Aerobic Compost (HTAC) Produced from Sewage Sludge for Rice Yield. International Journal of Biology, 10 (3), 2018, 27-38.
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