INFLUENCE OF PERCOLATION PATTERNS AND CONCENTRATIONS OF POLLUTED SOIL ON COPPER UPTAKE, AND GROWTH AND YIELD OF RICE PLANTS IN COPPER-POLLUTED STRATIFIED PADDY FIELDS

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BIOTIC ENVIRONMENT SCIENCE

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A Thesis Submitted

By

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Dedicated

To my Mother and Father

Abstract

Copper(Cu), arsenic, and cadmium(Cd) are designated as specific substances of the Agricultural Land Soil Pollution Prevention Act in Japan. It has been known that high Cu concentrations in soil layers reduce rice crop production and therefore agricultural practices such as soil dressing have been applied to minimize damage to crops by Cu pollution. In this study, we investigated the effects of percolation patterns of the plowsole and the subsoil on growth and yield, and Cu uptake of paddy rice. Six stratified paddy field models were constructed to conduct growth tests under the condition that the percolation patterns of plowsole and subsoil were in an open or closed system. These models had a plow layer and an upper plowsole made with 12.5cm-thickness of non-polluted soil dressing (3.7 mg/kg) and underlying 15cm-thickness of a polluted lower plowsole and a subsoil layer whose Cu concentrations were either higher (approximately 150，250，500mg/kg) or lower (approximately 40，70，100mg/kg) than Japanese safety standards (125mg/kg). The two percolation systems were applied to paddy field models by controlling the ground water level at 57.5 cm and 12.5 cm for the open system and closed system model respectively. During the tests, a constant water-ponding system was adopted, and mid-summer drainage was not done.

In open system percolation, the pressure head of the plowsole and subsoil were negative but in closed system showed positive pressure. The plow layer of O-40，O-70，O-100，O-150，O-250 and O-500 became reduction layers (under -100mV) while the plowsole and the subsoil became oxidation layers (over 400mV). On the other hand, Eh values of C-40，C-70， C-100，C-150， C-250 and C-500 were gradually decreased after transplanting, and in due time all the layers became reduction layers as Eh values showed under 0 mV. This means that, in this study, the polluted soil layers in O-40，O-70，O-100，O-150，O-250 and O-500 were under oxidation condition while those layers in C-40，C-70， C-100， C-150， C-250 and C-500 were under reduction condition.

As a result, Cu concentrations in the rice grains were 5% significantly higher in the open system percolation models regardless of the original amount of Cu in the plowsole and subsoil. On the other hand, we did not recognize the significant difference in growth and yield of rice plants among the models. We concluded that the Cu concentrations in rice plants were affected by percolation patterns of the polluted plowsole and subsoil even though they were covered with non-polluted soil dressing layers.

Key words: Copper, rice, percolation patterns, soil dressing, redox potential

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Chapter 1

Introduction

1.1 Heavy metals

The term "heavy metals" refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Lenntech, 2004). As a general term for metals and metalloids, heavy metal is applicable for the description of metals and metalloids with the atomic density larger than 4 g/cm^3 or 5 times or more compared with water (Hutton and Symon, 1986; Battarbee et al., 1988; Nriagu and Pacyna 1988; Nriagu, 1989; Garbarino et al., 1995, Hawkes, 1997). Nonetheless, heavy metal is significantly correlated with its chemical property instead of density. There are a large number of heavy metals, including zinc (Zn), arsenic (As), lead (Pb), cadmium (Cd), copper (Cu), mercury (Hg), silver (Ag) chromium (Cr), iron (Fe), and platinum(Pt). The overall condition of one organism or more organisms is referred as environment, which particularly represents the comprehensive external conditions affecting the existence and development of organisms (Farlex, 2005). The environment involves all animate and inanimate things, including terrestrial, aquatic, and atmospheric habitats. Some more tangible factors such as air, water and food, and some less tangible factors like social communities are discussed for the assessment of environment (Gore, 1997). Pollutants refer to the materials that result in adverse influences, impair the welfare of the environment, affect people's living standard or even lead to the death of living things. The government has strict regulations for substances with heavy metals in the environment, which should be within the tolerance limit, either a desirable or acceptable limit. As a result, there is a close correlation between environment and heavy metal pollutions. With the existence of toxic pollutants, living things will be affected by the bad external conditions, like posinous air, water and soil. With modern industrial development, there is increasing environment pollution. The emission of "three wastes" , urban living garbage, pesticides with heavy metals, and unreasonable use of chemicals result in the soils polluted by heavy metals. Thus , pollutants are accumulated gradually in humans and animals , resulting in damages to their health accordingly. Therefore, people are more and more concerned about heavy metal accumulation in agricultural productions and pollutant control measures (Zhang et al., 2007).

1.2 The origin of copper pollution in agricultural soil

After the first metal mine in Japan began operating in the early eighth century, metal mining became an established industry between the late sixteenth and early seventeenth centuries, and especially during the Edo period (1603–1867) when many metal mines were developed. In Meiji Period of Japan (1868–1912), metal industry achieved great development. At that time, large scale metal enterprises were established in Japan with the support of Japanese government and the modern management. To meet the development of general and military industries, there was a growing requirement for copper (Cu) and zinc (Zn), as the two metals could be used for civil and military purposes. In this context, there was a marked increase in the production of metals. With the conclusion of the World War II, the significant economic development also pushed forward the higher demand for various metals in the 1960s. Under such circumstance, people excavated numerous metal ores to satisfy the needs. However, the domestic production could not meet the fast growing needs, thus people had to import more metal ores from other countries. In this period of time, a large number of heavy metals were thrown away into the surrounding environment without treatment, resulting in extensive soil contaminations due to the pollutants, such as copper (Cu) (Tomohito et al., 2010).

1.3 Background of the study

The studies of Cu pollution in agricultural lands have mainly been focused on damage to crops such as growth inhibition, while Cd concentrations in brown rice itself have been another significant issue of soil pollution. The problem of soil Cu pollution has been of relatively small interest compared to Cd pollution. It is probably because Cu pollution rarely directly affects human health (Kobayashi, 1978, Takaishi et al., 2015) and the area affected by Cu pollution is not as large as that of Cd contamination in Japan. It is reported that in apple orchards, Bordeaux mixtures, mixture of copper sulphate and calcium carbonate have been used for a long time and thus soil Cu concentrations in some orchards are as high as several hundred mg/kg (dry soil) (Aoyama, 2009) while the safety standard of paddy soil Cu concentrations in Japan is 125 mg/kg. Since apple farming is really hard work, elderly farmers, especially, tend to abandon their orchards. Some of the apple orchards in lowland had once been converted from paddy fields and there is a possibility that they will be restored to paddy fields, which require less labor. Therefore, it should be necessary and important to develop the technique of minimizing Cu uptake of paddy rice plants.

1.4 Copper study in rice cultivation

In Japan, soil dressing has mainly been applied for remediation of Cu polluted soil (Asami, 2010). Recently, Paul et al. (2011a, b) and Sasaki et al. (2016a, b) clarified that variations of percolation patterns of the plowsole and the subsoil using stratified paddy field models with soil dressing layers resulted in significant differences in Cd concentrations in the brown rice. Paul et al. (2011b) also showed that the percolation patterns affected the amount of Cu accumulation in rice plants even though they used non-polluted soil (12.2 mg/kg). Since the solubility of Cu increases under the oxidation condition and decreases in the reduction condition as in the case of Cd (Dong et al., 2007), the percolation patterns in stratified paddy fields may affect the Cu uptake and growth and yields of rice plants. It has been reported that Cu polluted soil is likely to induce a Cu accumulation in the roots of paddy rice and a decrease in the number of panicles and the ratio of ripening (Chino et al., 1966; Shibuya, 1979). Shibuya (1979) also mentioned that the yields of brown rice had decreased by approximately 10% under the condition that Cu concentrations in the subsoil layer were higher than 200 mg/kg with a 15cm-thick soil dressing. These studies, however, did not consider the variation of percolation patterns.

1.5 Countermeasures against copper contamination of agricultural crops

In 1970, the law on the prevention of soil pollution in agricultural land was formulated. The law stipulates that copper, arsenic (As) and cadmium (Cd) are designated as specific pollutants. thus a variety of techniques for minimizing the heavy metals uptake of crops have been developed, for example, soil dressing, chemical measurement, phytoremediation, and breed improvement of rice plants. It has also been recommended to keep the soil in reduction condition by flooding during the whole growing period in order to reduce Cd and Cu uptake (Yamane et al., 1997; Asami, 2005 ; Inahara et al., 2007 ; Akahane et al., 2013).

1.6 The purpose of this study

The purpose of this study is to elucidate whether the percolation patterns affects the growth, yield and copper absorption of rice plants. The stratified paddy field model was prepared with about 40 mg/kg, 70 mg/kg, 100 mg/kg, 150 mg/kg, 250 mg/kg, and 500 mg/kg Cu contaminated soil, while copper safety standard (125 mg/kg). Therefore, the objectives of the study were as follows:

1. to investigate Cu uptake of rice plants from polluted soil affected by percolation patterns

2. to investigate the effects of soil Cu on growth and yield of paddy rice with open or close percolation pattern relationship between percolation patterns and paddy rice yield of the Cu polluted field.

The results showed that the percolation pattern significantly changed the concentration of Cu in brown rice, but did not affect its growth and yield.

Chapter2

Materials and methods

2.1 Introduction

Growing experiments of rice plants were done with stratified paddy field models. The experiment was conducted in the green house at Hirosaki University, Hirosaki-city, Aomori, Japan in 2016 and 2017. The stratified paddy field models have two modes: open system percolation and closed-system percolation. Pressure head is the criterion for open and closed-system. The closed and open-system percolation is often observed at the fields with poor drainage well drainage. respectively. We prepared stratified paddy field models with approximately 40 mg/kg, 70 mg/kg, 100 mg/kg, 150 mg/kg, 250 mg/kg and 500 mg/kg Cu contaminated soil.

2.2 Experimental Design and Materials

In this study, two types of stratified paddy field models were used for the experiment: the open-system percolation model and the closed-system percolation model. The percolation patterns were defined in Sasaki et al. (1992). Each stratified paddy field model was constructed in an iron box (30cm×50cm×70cm) filled with three layers of soil which was shown in Fig.1. The plow layer was from 0~10cm deep from the soil surface packed with non-polluted Kanagi soil (dry bulk density in puddling condition was 1.04 g/cm^3). The plowsole was from 10~20cm deep with non-polluted and polluted soil (dry bulk density from 10~12.5cm [non-polluted Kanagi soil] and from 12.5~20cm deep [Cu mixed polluted Bunkyou soil] were 1.23 g/cm^3 and 0.75 g/cm^3, respectively). The subsoil was from 20~55cm deep with polluted Bunkyou soil and non-polluted gravel (dry bulk density at the depth from 20cm to 27.5cm [polluted Bunkyou soil] and from 27.5cm to 55cm [the gravel] was 0.75 g/cm^3 and 1.40 g/cm^3, respectively.) These subsoil layers were formed by compaction. Hereafter, the models are called O-40 ,C-40, O-70, C-70, O-100 ,C-100, O-150 ,C-150, O-250 ,C-250 and O-500 ,C-500,where the numbers 40, 70, 100, 150, 250 and 500, mean soil Cu concentration as 49, 71, 95, 155, 247 and 519 mg Cu/kg, respectively. ('O' and 'C' stand for the open-system and the closed-system percolation, respectively). The ground water levels of the open-system and the closed-system percolation models were controlled at 57.5cm and 12.5~20cm depth, respectively. In the closed-system percolation models, the holes of the side walls of iron box were blocked in order to prevent the aeration. On the other hand, in the open-system percolation models, the holes of the side walls of the iron box were open in the lower part of the plowsole and the upper part of the subsoil in order to aerate those layers.

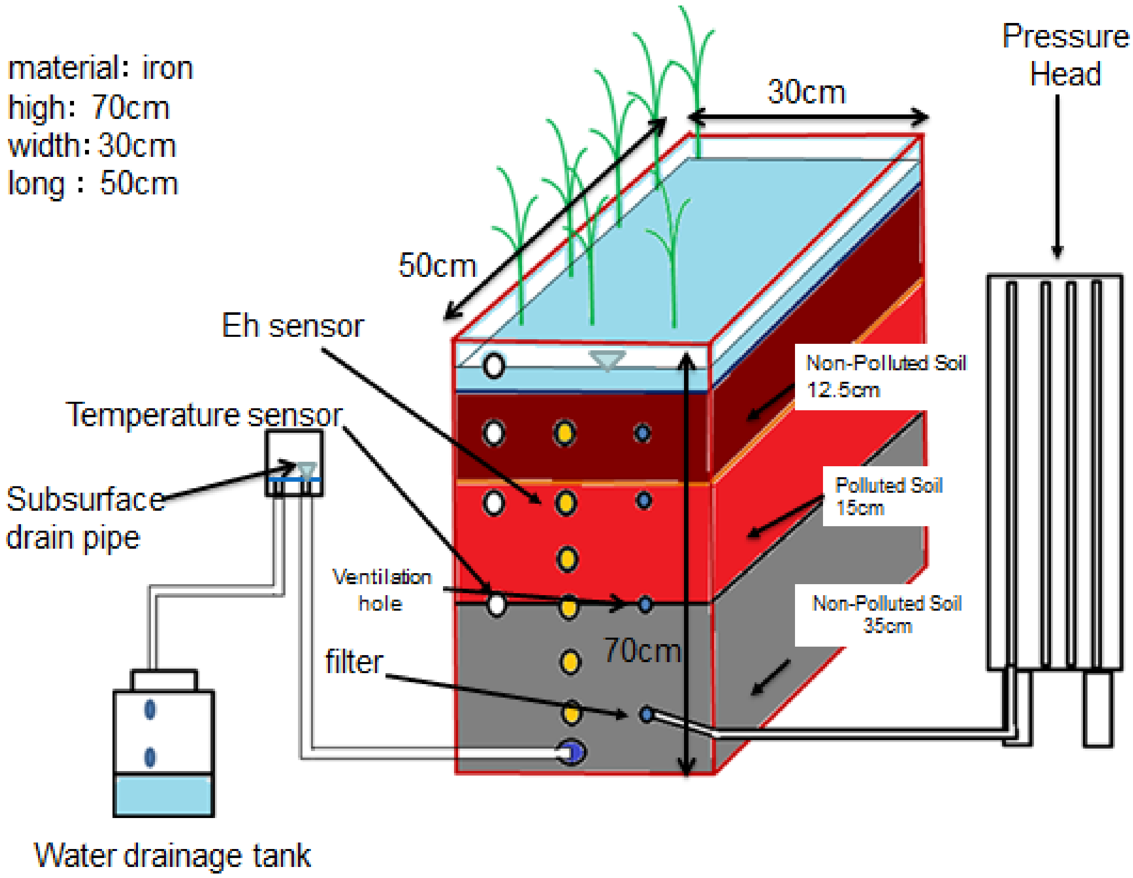


Fig.1 Experimental device

Table 1 shows the physical and chemical properties of the soils used in this study. Kanagi soil (Loam), 3.7 mg/kg Cu concentration, was sampled from a plow layer of the paddy field in Kanagi farm of Hirosaki University, Aomori prefecture. Bunkyou soil (Clay Loam) was made by adding a solution of $CuCl_2 \cdot 2H_2O$ to the soil which had been sampled from a plow layer of the paddy field on Bunkyou campus of Hirosaki University, Aomori prefecture, and both were mixed well. Cu concentrations in the soil of Bunkyo campus were originally 10.5 mg/kg. We produced six levels of Cu contaminated soil, Cu concentrations in which were either lower (49 mg/kg, 71 mg/kg, 95 mg/kg) or higher (155 mg/kg, 247 mg/kg, 519 mg/kg) than Japanese safety standard (125 mg/kg). These values are 10, 15, 20, 30, 50 and 100 times, respectively, as large as the average Cu concentration of non-contaminated paddy fields in Japan (4.47 mg/kg) (Asami, 2010). The organic matter (OM) content of the Kanagi soil(latitude:40°54′8″ , logitude:140°28′2.9″) and Bunkyou soil (latitude:40°35′9.6″ , logitude:140°28′28.2″) was 4.7% and 6.6%, respectively. The gravel, which contained 0.8 mg/kg of Cu, was used for the lower layer of the models since they were designed after the fashion of paddy fields near a river.

Table 1 Physical and chemical properties of soil samples and gravel

	Density (g/cm^3)	Soil Texture	MgO	CaO	K_2O	Cu	T-C (%)	T-N (%)	C/N	OM (%)
			(mg/kg)							
Kanagi Soil	2.58	L	229	531	306	3.7	2.74	0.18	15.40	4.7
Bunkyou Soil	2.61	CL	219	1,848	373	10.5	3.84	0.26	14.50	6.6
Gravel	2.68	-	-	-	-	0.8	0.04	0.01	4.00	0.1

Note: Soil texture is based on the International Union of Soil science classification; CL: Clay loam. Gravel diameter size 2-4mm; *mg /kg.

2.3 Preparation of soil

The preparation of soil requires four steps.

1. After collecting the soil, remove the plant debris and small stones from the soil as shown in Fig.2.

2. Mix the soil in the bowl with water make dirt balls of 10-12 cm in diameter as shown in Fig.3.

3. The balls are kept in an open place to dry, as shown in Fig 4.

4. The balls are then smashed with a mallet and sieved with 4.75mm diameter to make their size suitable for making soil layers (as shown in Fig 5, 6 and 7).

Fig.2 Remove the plant debris and small stones

Fig.3 Preparation of soil ball

Fig.4 Drying of soil ball

Fig.5 Smash dry balls

Fig.6 Crash with the mallet

Fig.7 Sieved of smashed balls

2.4 Preparation of soil layer

Each model was practiced in the iron box(30x50x70cm). Both of the percolation patterns, the dry bulk density of plow layer, plowsole, subsoil (soil) and subsoil (Gravel) were 1.04 g/cm^3, 0.75-1.0 g/cm^3, 0.75-1.0 g/cm^3, and 1.40 g/cm^3 and for the impermeable layer was 1.23 g/cm^3 as shown in Fig.8. The construction procedure of both models were four steps.

1. Fill the iron box with gravels with a thickness of 14cm (1.40 g/cm^3) from the bottom.(Fig.9)
2. Place the prepared Polluted soil on the top of the gravel (15cm-thickness, 0.75-1.0 g/cm^3) (Fig.10)
3. Put iron plate on Polluted soil and place non-Polluted soil(2.5cm-thickness, 1.23g/cm^3) on the iron plate. (Fig.11, Fig.12)
4. Place 10cm (1.04g/cm^3) thickness of non- Polluted soil on impermeable layer.

	depth/density (g/cm^3)	Constituent substance	open-system	closed-system
Water	7.5cm	water		
Plow layer	10.0cm(1.04)	Non-polluted soil	●	●
Plowsole	2.5cm(1.23)	Non-polluted soil	Impermeable layer	
	15.0cm(0.75-1.0)	Polluted soil	○	●
Subsoil (soil)			○	●
			○	●
Subsoil (Gravel)	35.0cm(1.40)	Gravel	○	●
			●	●

○ open system
● closed system

Fig.8 Soil layers of stratified paddy field model

Fig.9 Gravel layer

Fig.10 Prepared polluted soil

Fig.11 Iron plate on polluted soil

Fig 12 Non-Polluted soil on the iron plate (plowsole)

The groundwater level of open and closed system percolation models were kept at 57.5cm and 12.5cm depth, respectively. The groundwater level L(cm) was calculated with the equation L=H-h where H(cm) is the total soil depth and h(cm) is the distance between the bottom and soil layer and groundwater table (Fig.13).

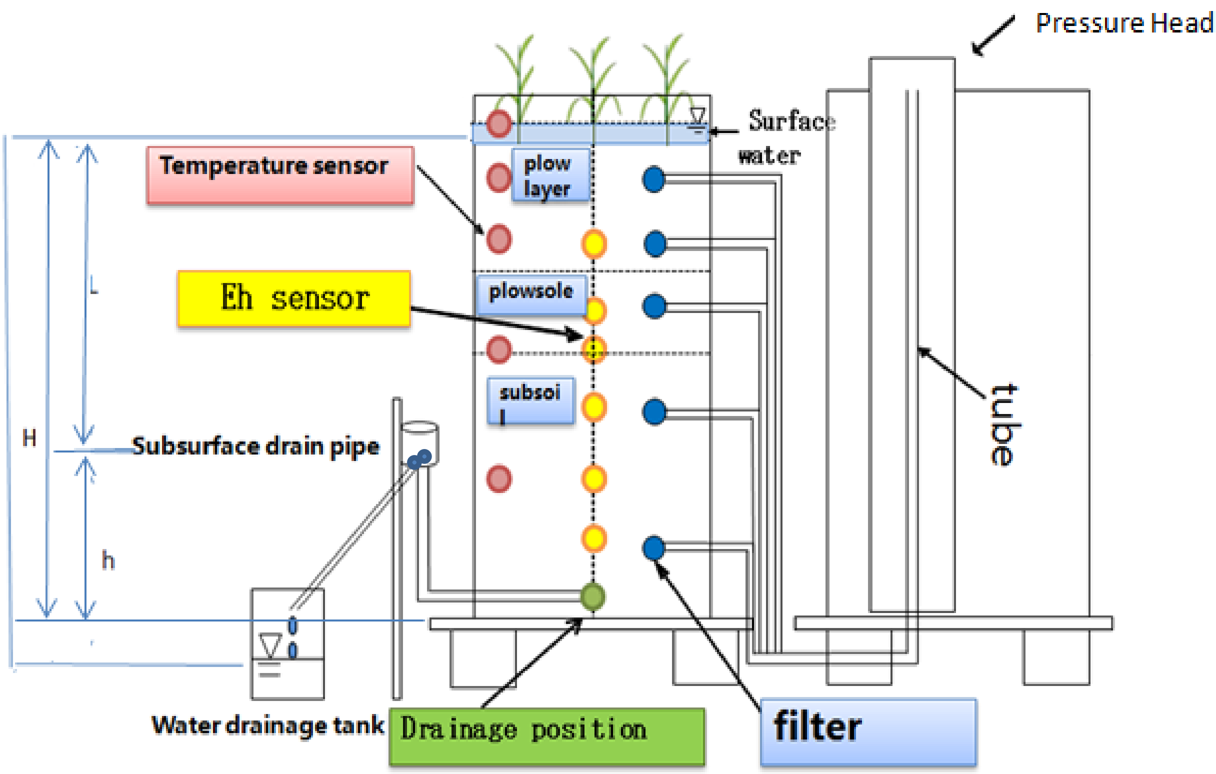


Fig.13 Layout of the experimental device

Eh and temperature sensors were inserted each soil layer for measuring soil physical and water condition as shown in Fig.14. The sensors inserted in each soil layer as Surface water (one temperature sensor), plow layer (one temperature and Eh sensor), plowsole (one temperature and two Eh sensors) and the subsoil (one temperature and three Eh sensors) as shown in Fig.15.

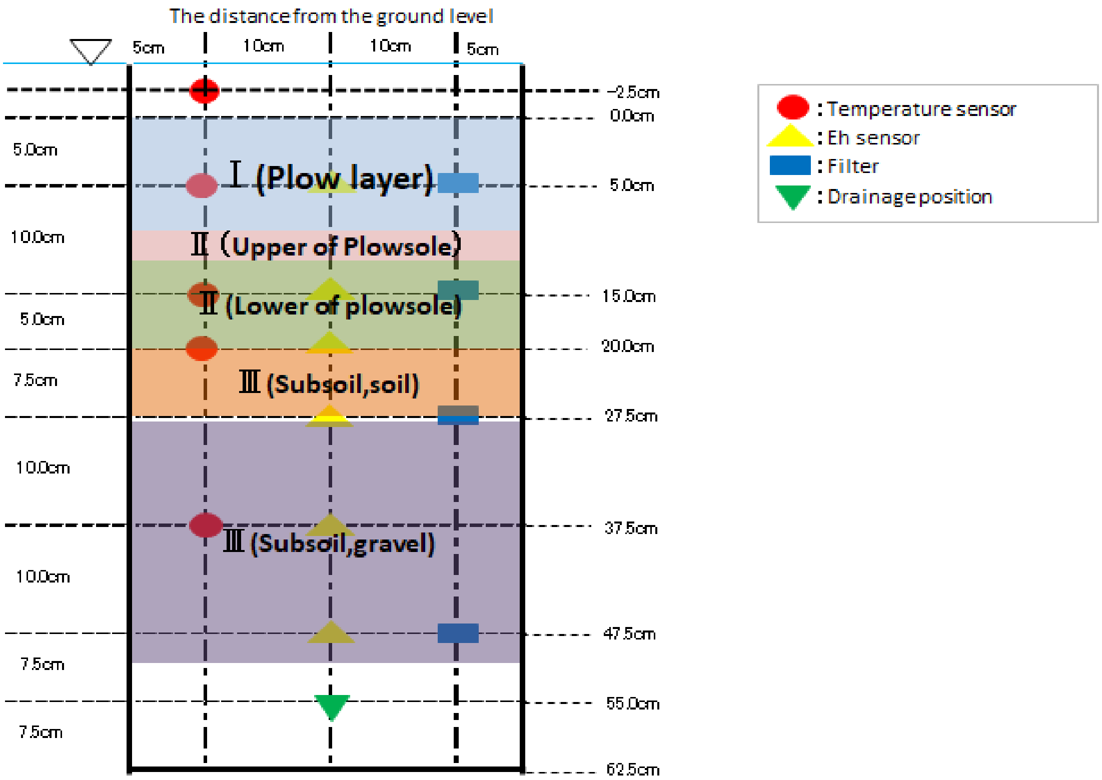
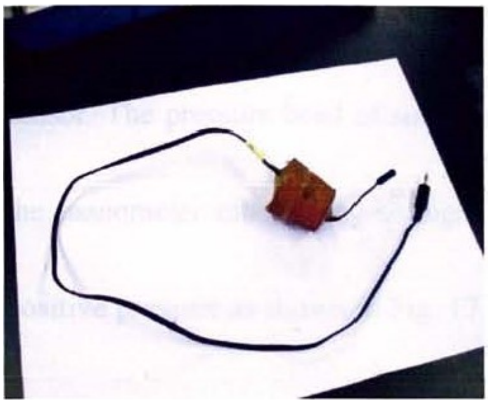
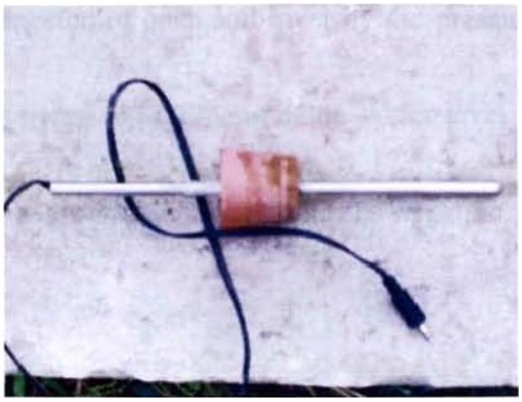


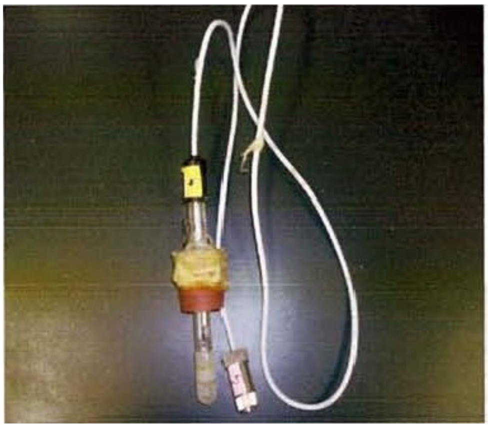
Fig.14 Gross-section View of sensor position and setting

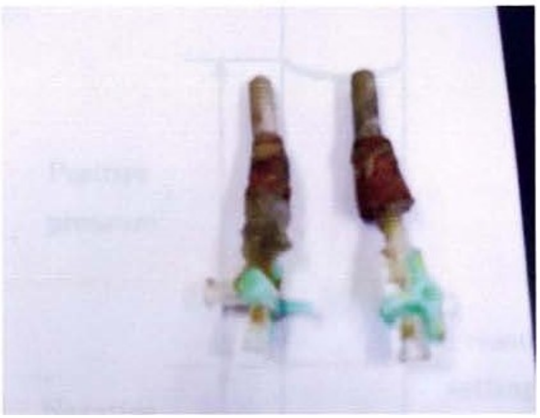




a) Temperature sensor (Type A)

b) Temperature sensor (Type B)





c) Eh electrode

d) Filter

e) Temperature recorder

f) ORP data meter

Fig.15 Sensors, Temperature recorder and ORP data logger

The pressure head distribution tubes were connected to each soil layer with suction cups. The pressure head of each soil depth was measured with manometer. When the water level in the manometer tube was below or above the groundwater level, it means that the soil water pressure in negative or positive, respectively (Fig.16, Fig.17).

Fig.16 Manometer board

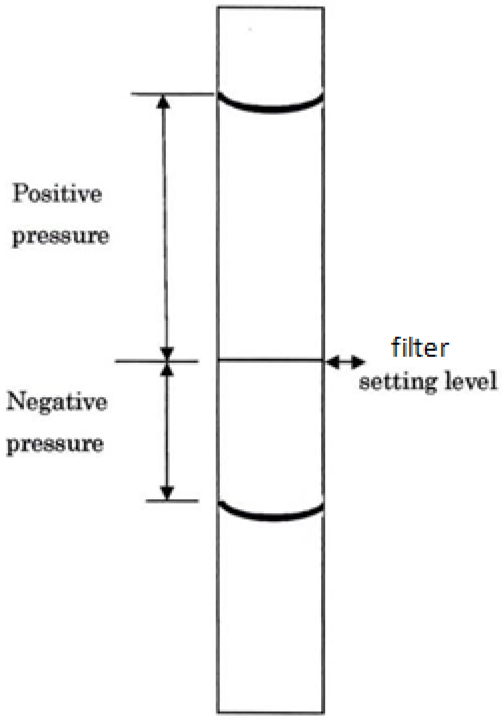


Fig.17 Definition of water pressure soil

2.5 Design of transplanting and Application of fertilizer

As for fertilizer, 2g of N, 2g of P_2O_5 and 2g of K_2O were applied per model and mixed with the whole plow layer before transplanting. Then after the models were prepared, fifteen paddy rice seedlings (the plant length and the leaf stage were from 12.5 to 17.5 cm and from 4.4 to 5.0 leaves, respectively) named '*Oryza sativa* L., Tsugaru Roman' were transplanted by 10cm intervals. During the cultivation period, the water ponding condition was constantly adopted and the mid-summer drainage was not done. Transplanting of the paddy seedlings and harvesting were conducted at the end of May(May 23, 2016 and May 25, 2017) and at the end of September (September 19, 2016 and September 20, 2017), respectively. The experiment with the stratified paddy field models was conducted in a greenhouse at Hirosaki university. The design of transplanting in the model was shown in Fig.18.

Fig.18 Design of transplanting into iron box and schematic diagram

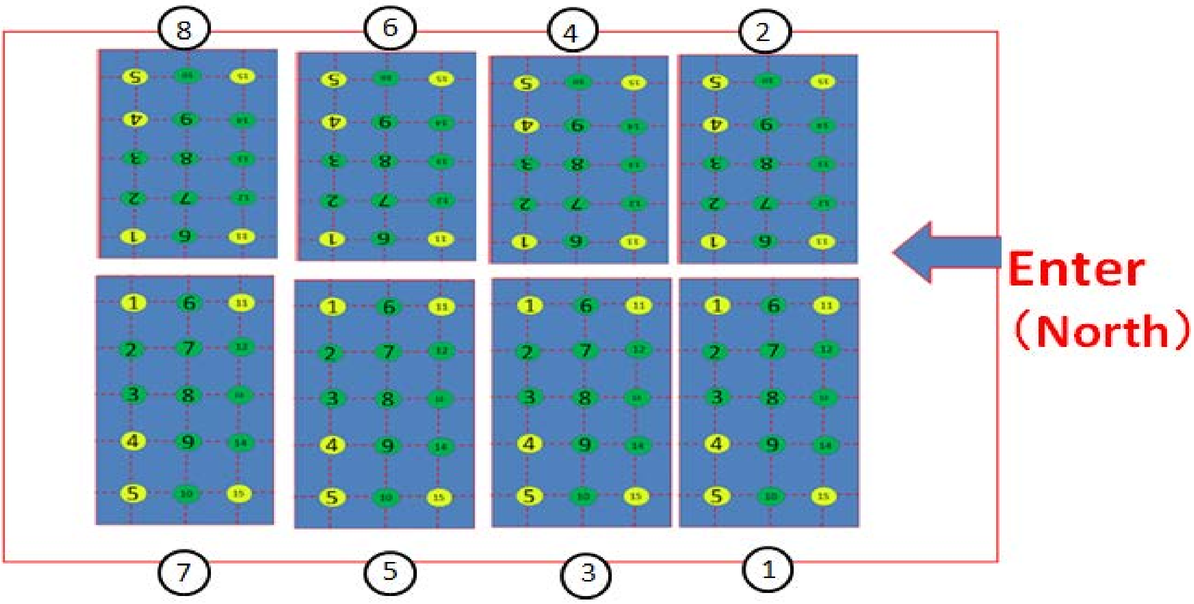


Fig.19 The rice configuration diagram in the model

2.6 Measurement methods

The examination of growth and yield of rice plants such as plant length, leaf stage, the number of stems and panicles, the weight of straw, the number of grains of brown rice and the weight of brown rice was done by the standard method of Iwate Agricultural Experimental Station (1981). The quantitative analysis of Cu concentrations in leaves, root, brown rice and soils extracted by HCl solution was carried out with atomic absorption spectroscopy (MAFF, 1979). Other measurements of soil physical and chemical properlies were also conducted in standard methods used in Japan. The Oxidation-Reduction Potential (ORP) meter (Central Kagaku Co. Ltd., model UC-203) was used for measuring ORP (Eh).

Chapter3

Results and Discussion

3.1 Introduction

In this experiment, we investigated the effects of percolation patterns of the plowsole and the subsoil on growth and yield, and Cu uptake of paddy rice under different levels of Cu soil pollution. Four stratified paddy field models were constructed to conduct growth tests under the condition that the percolation patterns of plowsole and subsoil were in an open or closed system. These models had a plow layer and an upper plowsole made with 12.5cm-thickness of non-polluted soil dressing (3.7 mg/kg) and underlying 15cm-thickness of a polluted lower plowsole and a subsoil layer whose Cu concentrations were either higher (approximately 250mg/kg) or lower (approximately 70 mg/kg) than Japanese safety standards (125 mg/kg). During the experiment, a constant water-ponding system was adopted, and mid-summer drainage was not done.

3.2 Day water loss in depth

Irrigation water is very important for growth and yields of rice plants. In this study, irrigation water was continuously supplied. The water requirement rate was controlled by the impermeable layer of both percolation systems. The average Day water loss in depth of models O-40, C-40, O-70, C-70, O-100, C-100, O-150, C-150, O-250, C-250, O-500 and C-500 were 8.7, 10.7, 10.4, 12.3, 8.2, 10.6, 8.3, 11.8, 9.0, 14.0, 8.0 and 8.8 mm/day, respectively, as shown in Fig.19 and Fig.20. The results show that the water loss of closed -system was larger than that of open-system. The average of water requirement rate was low at the beginning and the end of the experiment, while it became higher in the middle of the experiment period, from July to August.

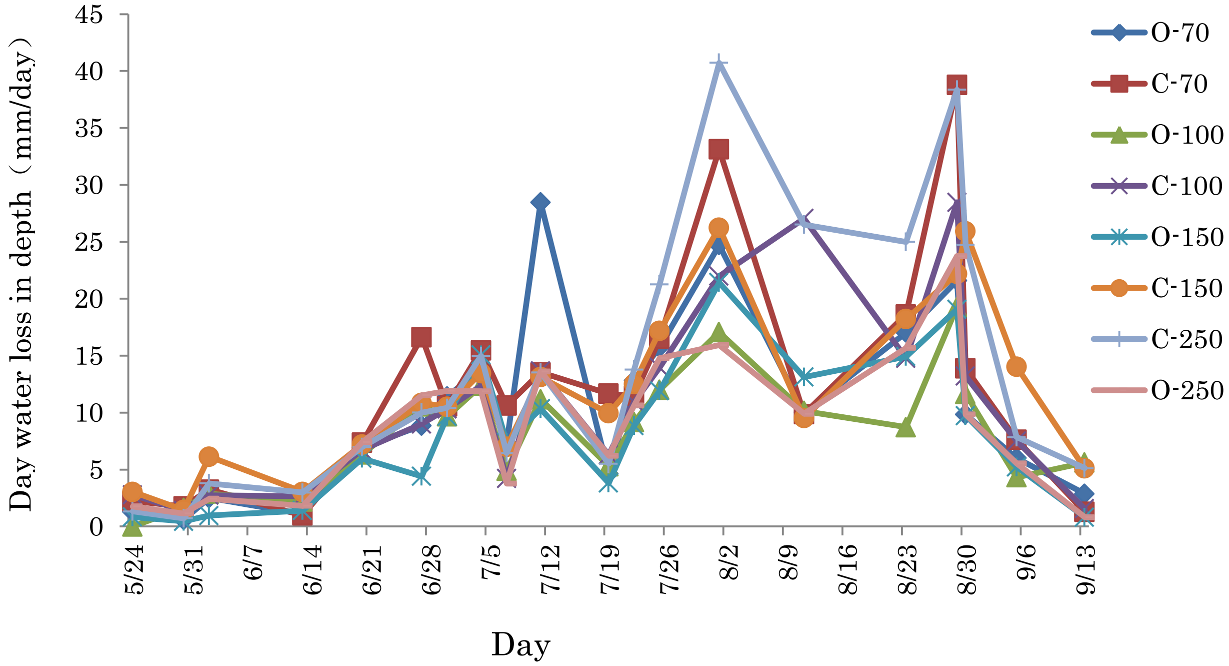


Fig.20 Day water loss in depth rate in stratified paddy field model under open and closed system percolation in 2016

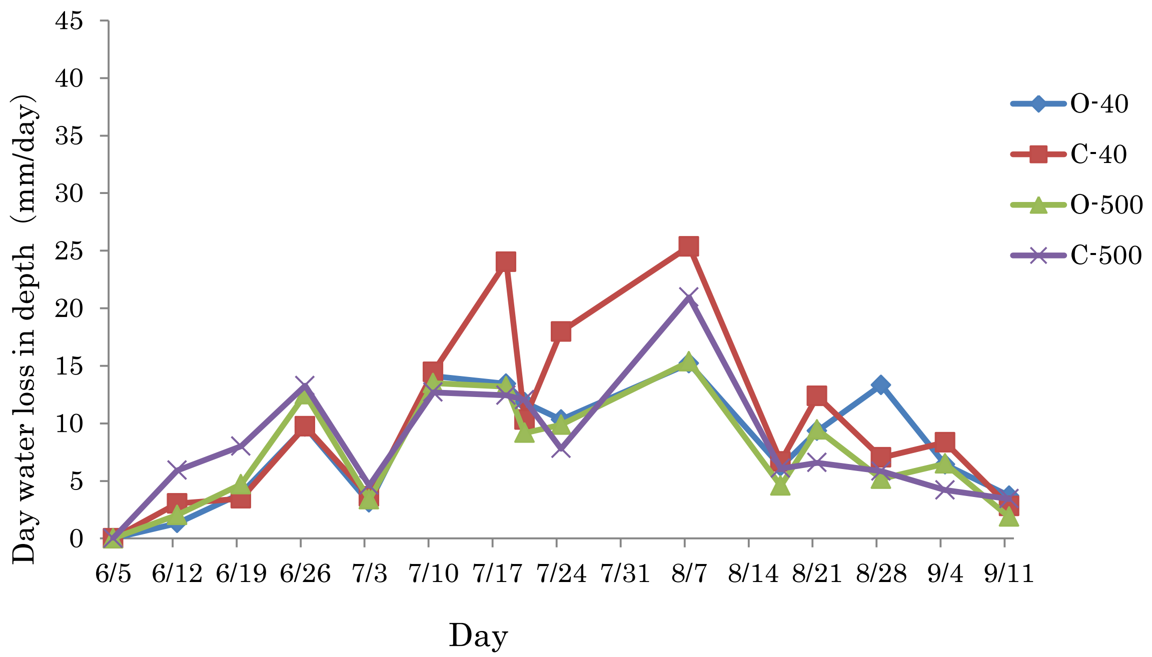


Fig.21 Day water loss in depth rate in stratified paddy field model under open and closed system percolation in 2017

3.3 Pressure head distribution

In 2016 experiment, the open system percolation (O-70, O-100, O-150 and O-250) showed positive pressure in plow layer but plowsole and subsoil were negative pressure due to unsaturated condition of those soil layers and air enter into those soil layers and in additional water pressure of plowsole and subsoil layers were lower than the air entry pressure (-2.0 cm) in agreement with the previous study by Sasaki (1994). It indicated that those layers were open system percolation. On the other hand, closed system percolation (C-70, C-100, C-150 and C-250) showed positive pressure of plow layer, plowsole and subsoil at the soil depth of 5 cm, 15 cm, 27.5 cm, 47.5 cm respectively due to saturated condition of those soil layer by water that shown in Fig. 22.

In 2017 experiment, the Model of O-40 and O-500 which were oriented as open system percolation pattern and showed positive pressure in plow layer but negative pressure in plowsole and subsoil. On the other hand, closed system percolation (C-40 and C-500) showed positive pressure of plow layer, plowsole and subsoil due to saturated condition by water as shown in Fig.23. Sasaki et al., (2001) described that positive pressure of soil layer were performed in closed percolation pattern and negative pressure in an open system percolation which is supported this study. Positive pressure of soil layer performed by ill- drained paddy field and negative pressure by welldrained paddy field due to water movement from higher potential to the lower point as described Hillel et al., (1998). The total potential of water pressure in the open system percolation was nearly equal to the pond level in plow layer. The high loss of water potential occurred in plowsole and subsoil had water loss in proportion with depth. The total potential of water pressure in plowsole and subsoil of open system percolation were less than the closed system percolation but total potential loss was less than that of the open system percolation due to the condition of plowsole.

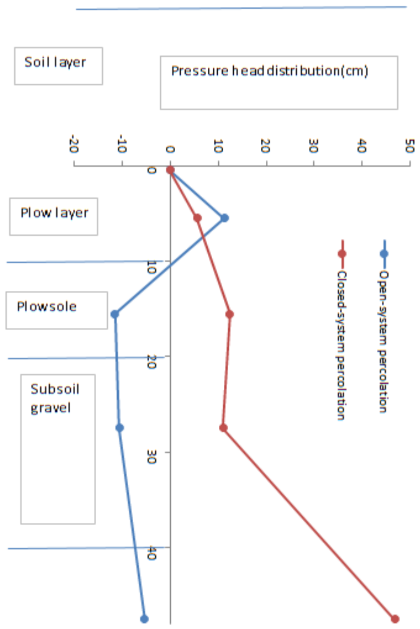


Fig.22 The pressure head profile in open (O-100) and closed (C-100) system percolation at 50th day-2016

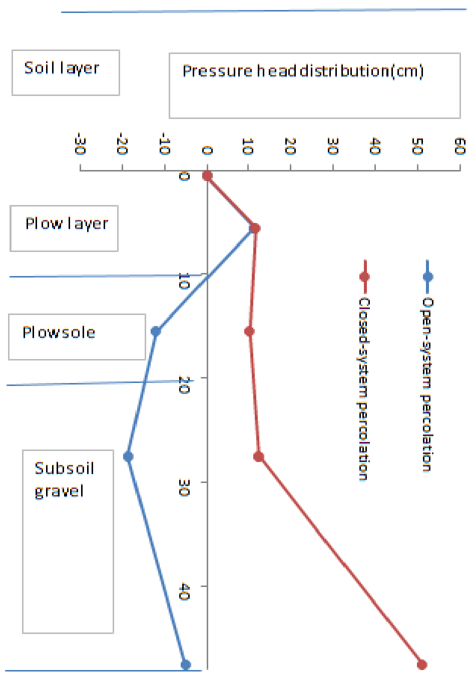


Fig.23 The pressure head profile in open (O-500) and closed (C-500) system percolation at 50th day-2017

3.4 Temperature

The leaf, stems and roots growth of rice plants depend on many factors. Low soil temperature during the growing season may cause substantial reductions in the growth of plants in agronomic and native ecosystems.

In this study, different depths of soil temperature were recorded from sowing to harvesting of rice. Air temperature was also measured of the green house in both years. The average high and low temperature recorded in the green house were 28.0^{o}C and 18.3^{o}C respectively. The temperature of greenhouse was 2~3^{o}C higher than the average temperature of Hirosaki city. The temperature into green, the average temperature of Hirosaki city and soil temperature of different depths are given in Fig.21. Soil temperatures of different depth for 2016 are shown in Fig.22~29. Those for 2017 are shown in Fig.30~33.

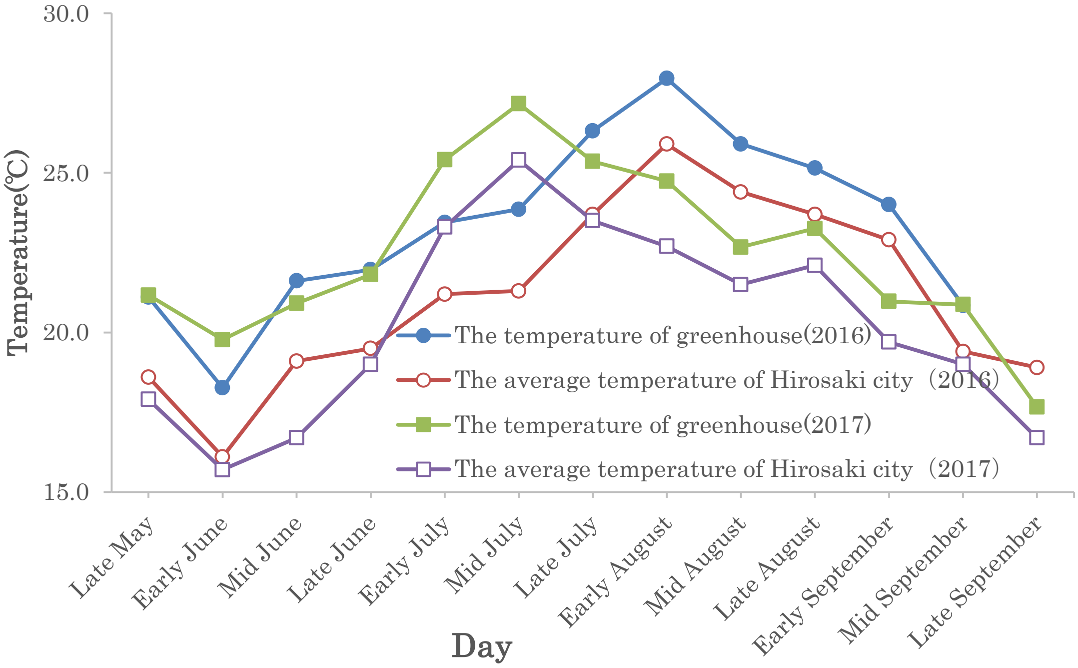


Fig.24 Temperature in the green house and the average temperature of Hirosaki city in2016 and 2017

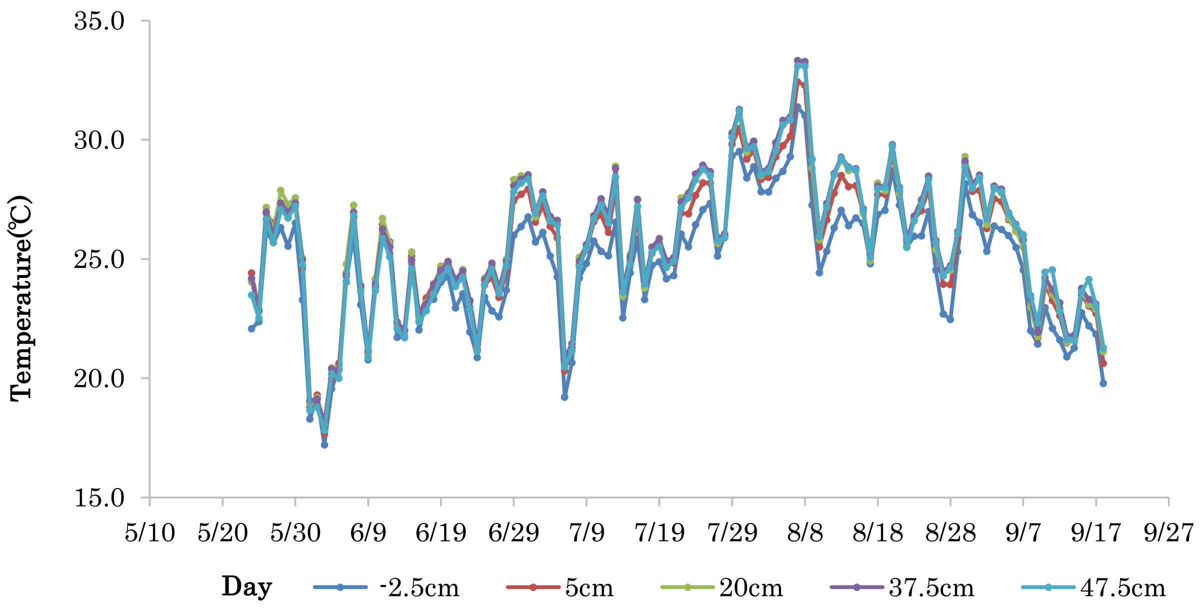


Fig.25 Temperatures of 2.5cm, 5cm, 20cm, 37.5cm and 47.5cm depth from soil surface in 70 mgCu/kg open system percolation models in 2016

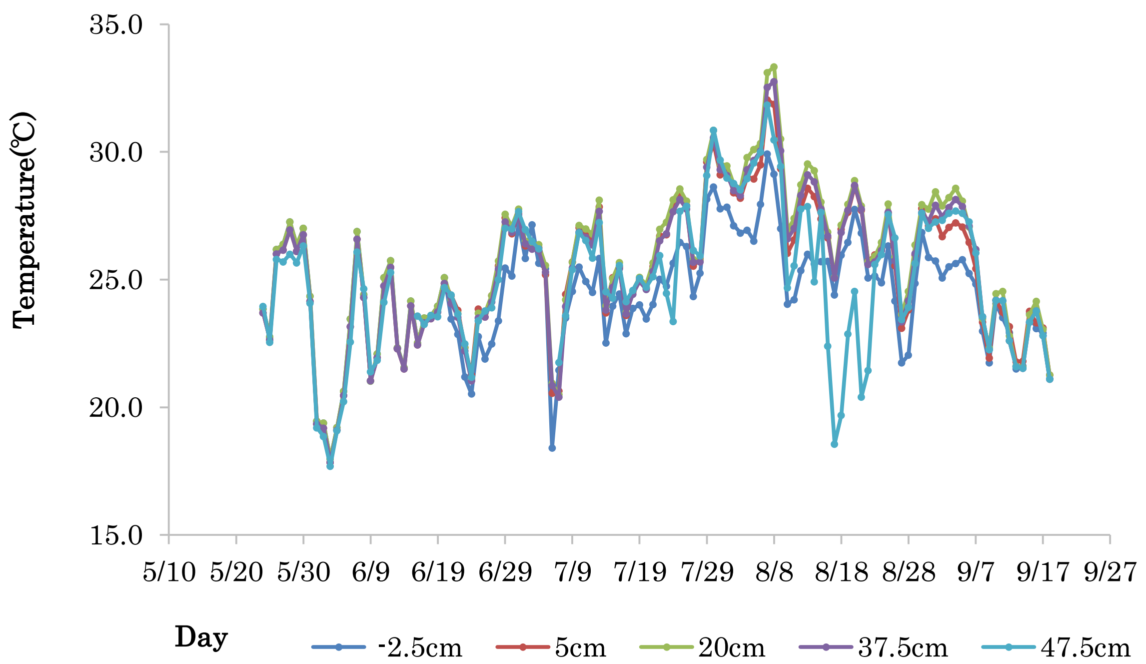


Fig.26 Temperatures of 2.5cm, 5cm, 20cm, 37.5cm and 47.5cm depth from soil surface in 70 mgCu/kg closed system percolation models in 2016

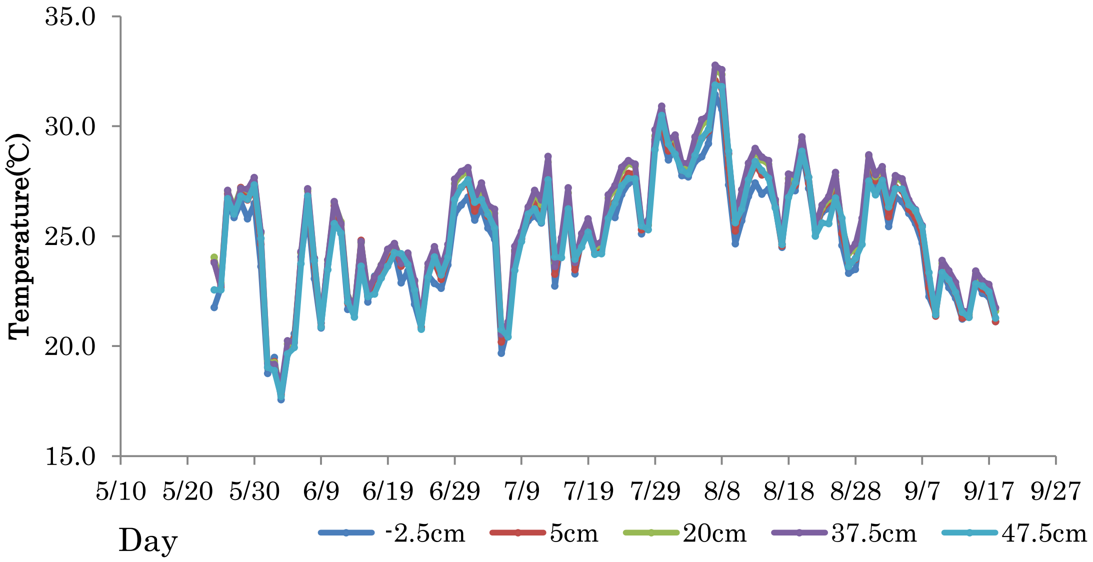


Fig.27 Temperatures of 2.5cm, 5cm, 20cm, 37.5cm and 47.5cm depth from soil surface in 100 mgCu/kg open system percolation models in 2016

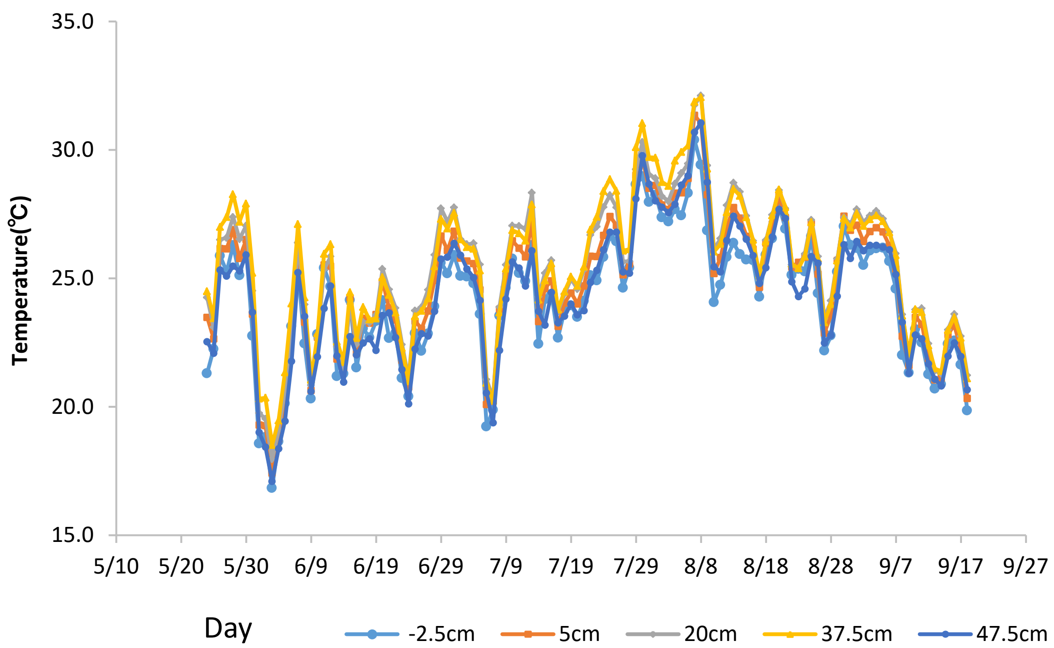


Fig.28 Temperatures of 2.5cm, 5cm, 20cm, 37.5cm and 47.5cm depth from soil surface in 100 mgCu/kg closed system percolation models in 2016

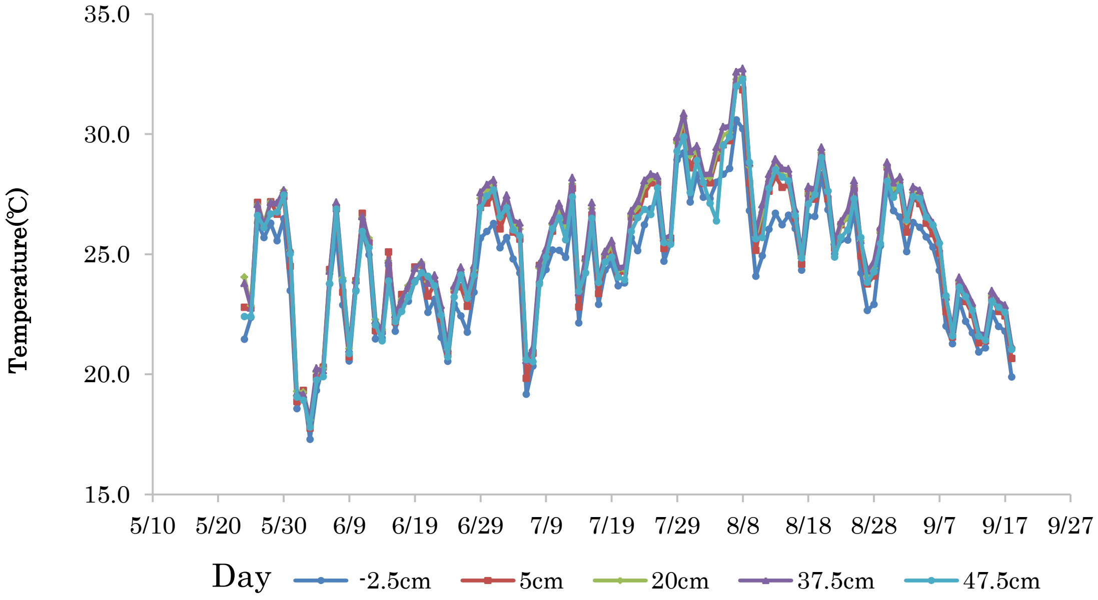


Fig.29 Temperatures of 2.5cm, 5cm, 20cm, 37.5cm and 47.5cm depth from soil surface in 150 mgCu/kg open system percolation models in 2016

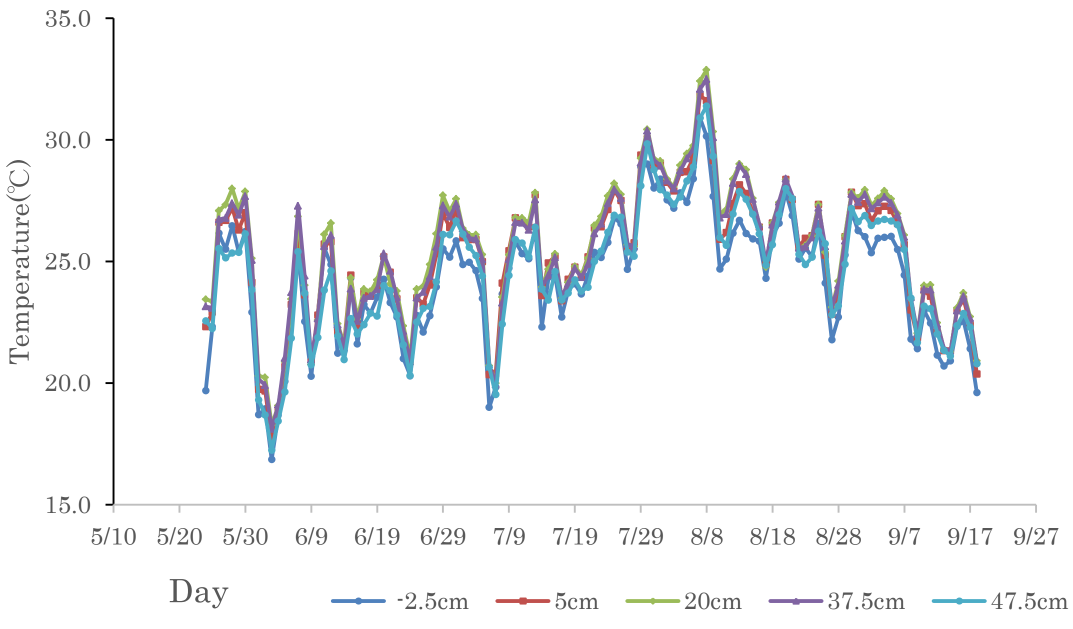


Fig.30 Temperatures of 2.5cm, 5cm, 20cm, 37.5cm and 47.5cm depth from soil surface in 150 mgCu/kg closed system percolation models in 2016

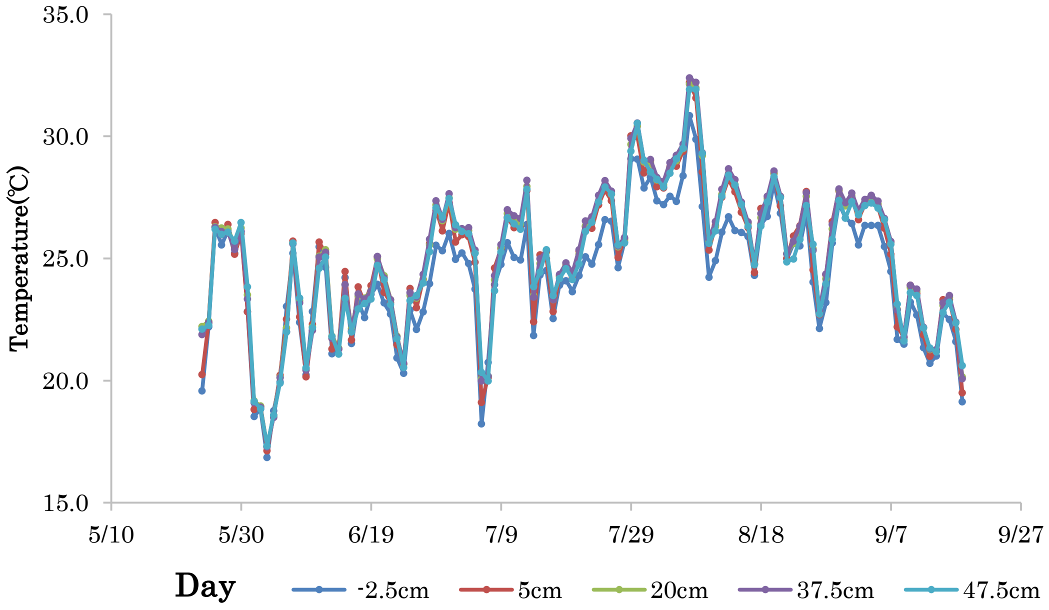


Fig.31 Temperatures of 2.5cm, 5cm, 20cm, 37.5cm and 47.5cm depth from soil surface in 250 mgCu/kg open system percolation models in 2016

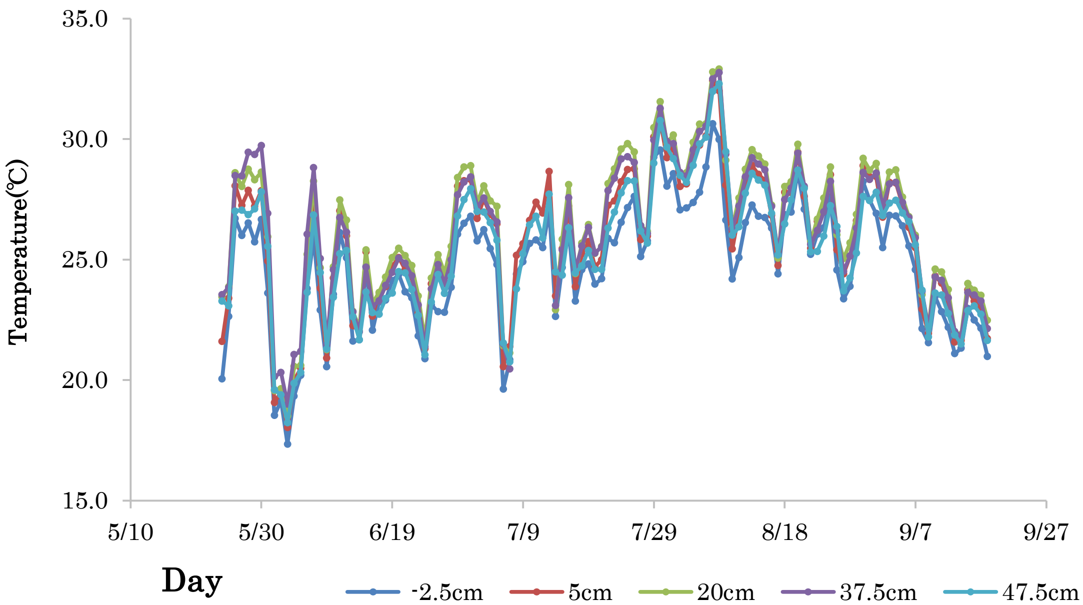


Fig.32 Temperatures of 2.5cm, 5cm, 20cm, 37.5cm and 47.5cm depth from soil surface in 250 mgCu/kg closed system percolation models in 2016

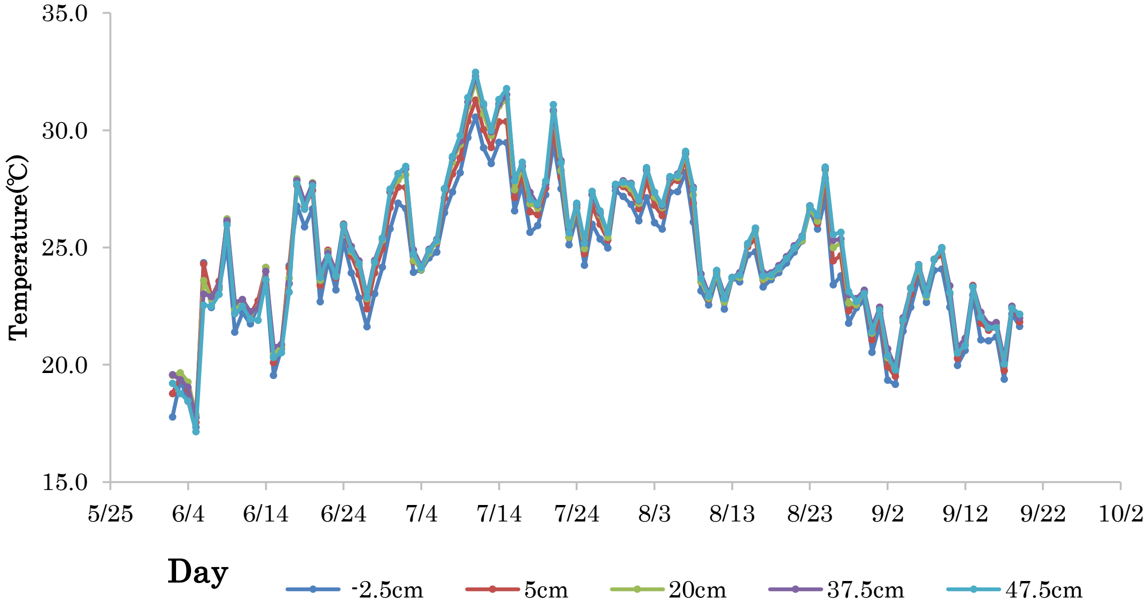


Fig.33 Temperatures of 2.5cm, 5cm, 20cm, 37.5cm and 47.5cm depth from soil surface in 40 mgCu/kg open system percolation models in 2017

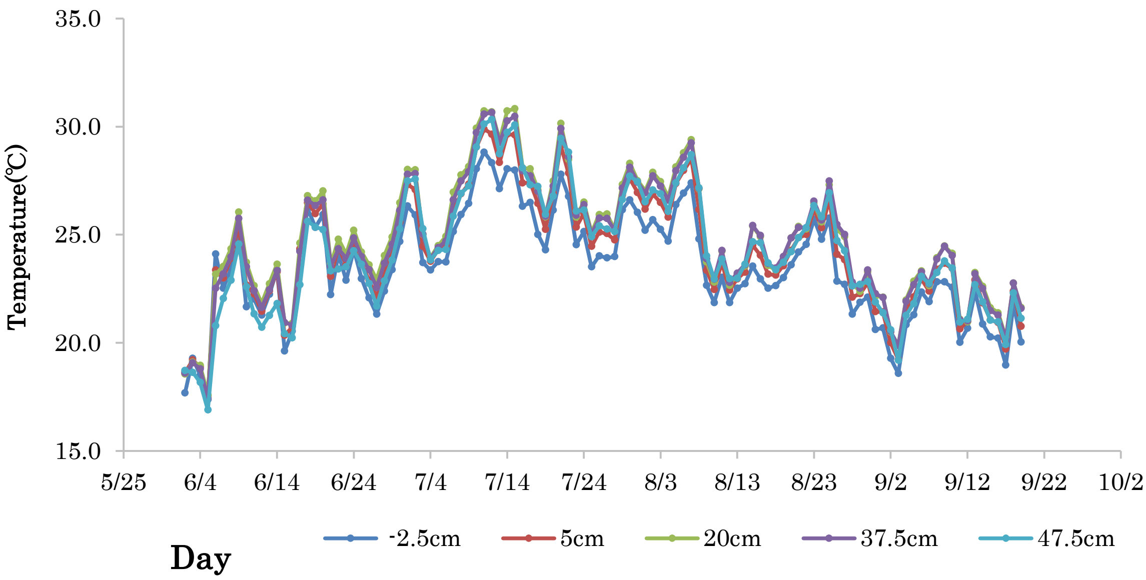


Fig.34 Temperatures of 2.5cm, 5cm, 20cm, 37.5cm and 47.5cm depth from soil surface in 40 mgCu/kg closed system percolation models in 2017

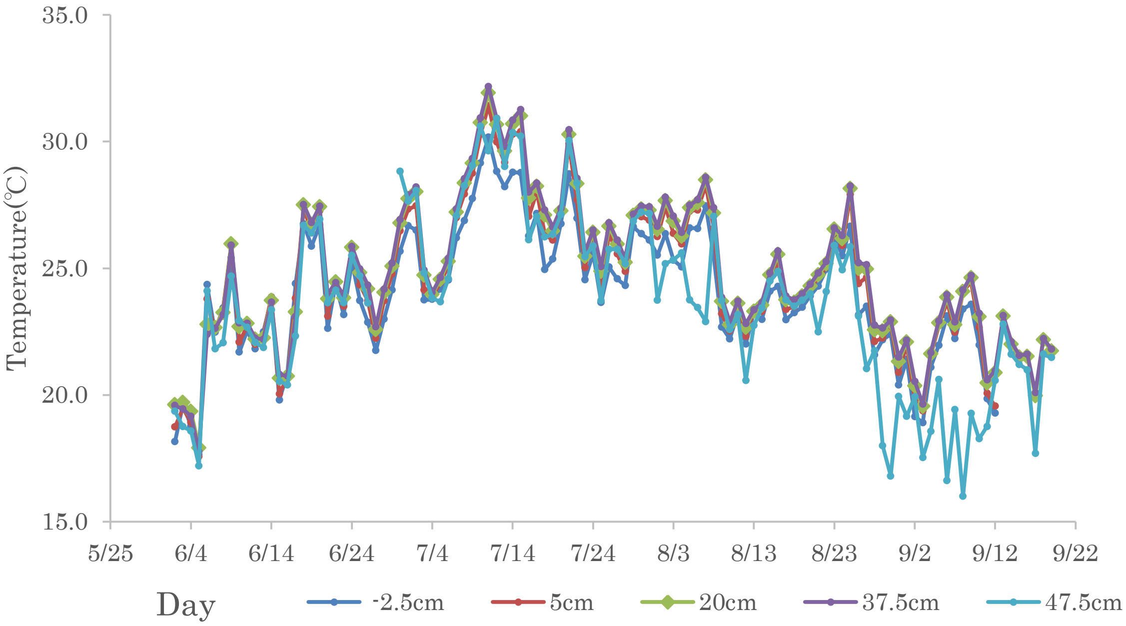


Fig.35 Temperatures of 2.5cm, 5cm, 20cm, 37.5cm and 47.5cm depth from soil surface in 500 mgCu/kg open system percolation models in 2017

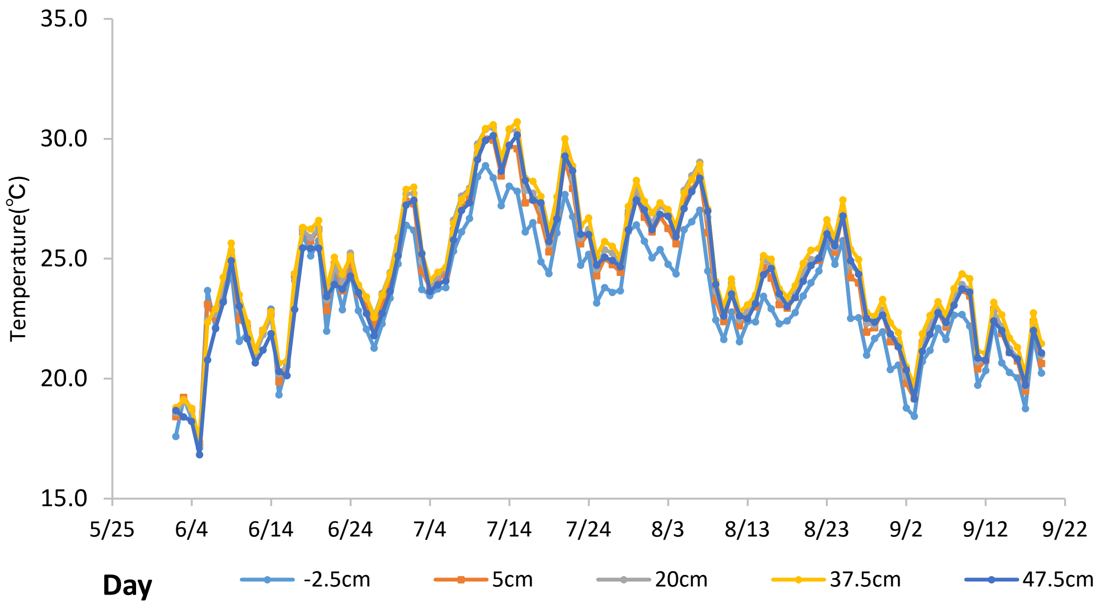


Fig.36 Temperatures of 2.5cm, 5cm, 20cm, 37.5cm and 47.5cm depth from soil surface in 500 mgCu/kg closed system percolation models in 2017

3.5 Oxidation redox potential (Eh)

The temporal changes of Eh are shown in Fig.34~45. The plow layer of O-40, O-70, O-100, O-150, O-250 and O-500 became reduction layers (under -100mV) while the plowsole and the subsoil became oxidation layers (over 300mV). On the other hand, Eh values measured at the depths of C-40，C-70，C-100，C-150，C-250 and C-500 were gradually decreased after transplanting, and in due time all the layers became reduction layers as Eh values showed under 0 mV. This means that, in this study, the polluted soil layers in O-40，O-70，O-100，O-150，O-250 and O-500 were under oxidation condition while those layers in C-40，C-70，C-100，C-150，C-250 and C-500 were under reduction condition. It has been pointed out that the Cu uptake in rice is affected by the oxidation-reduction environment (Matsunaka, 2014) and, therefore, in this study, Cu solubility was probably high in the models of O-40，O-70，O-100，O-150，O-250 (Takaishi et al., 2015). We decided on the oxidation and reduction condition on the basis of Yamane (1982), who had defined the oxidation layer as Eh value as 300mV or more and reduction layer as < 300 mV.

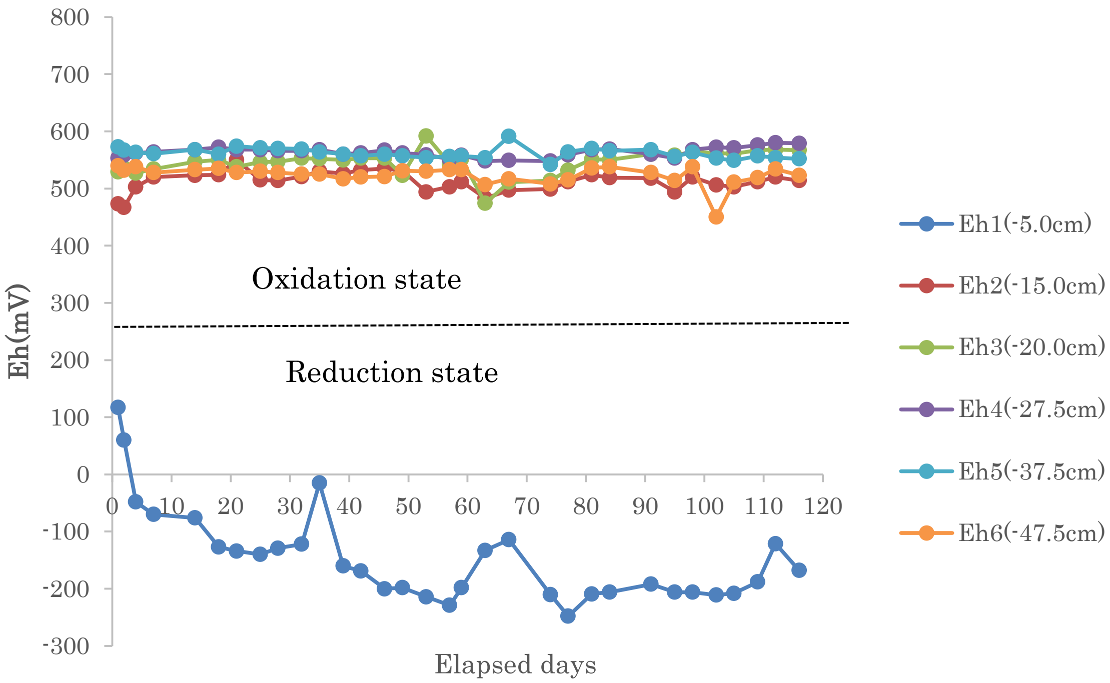


Fig.37 The temporal changes of Eh in the stratified paddy field model in 2016 (O -70)

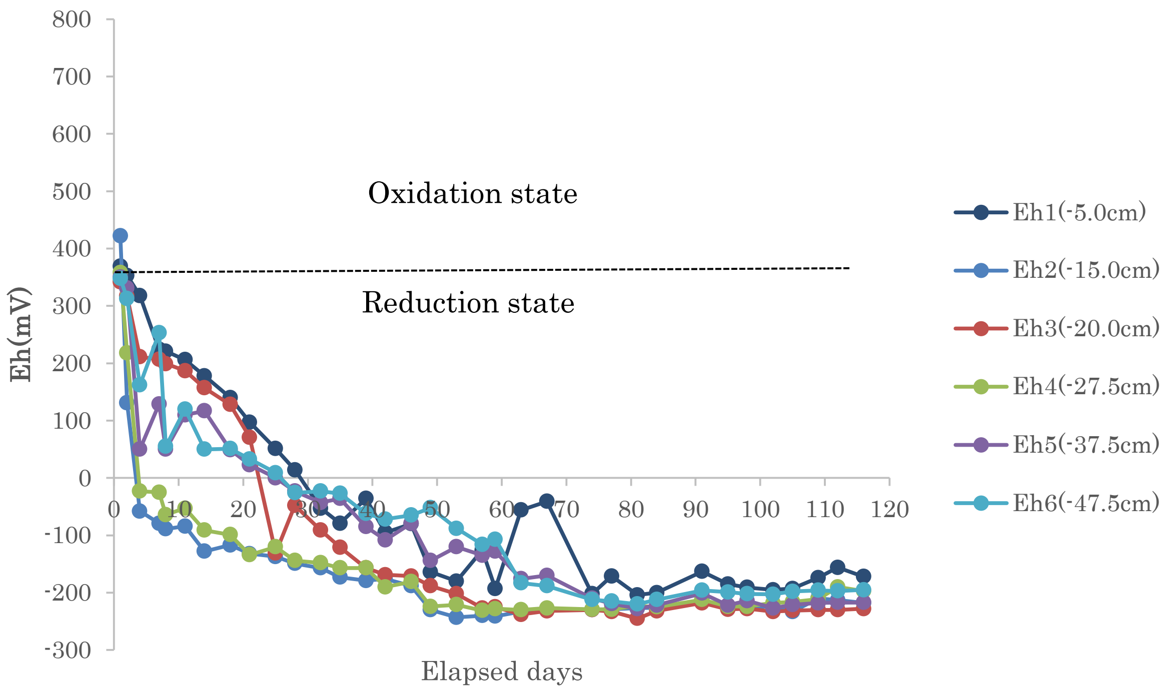


Fig.38 The temporal changes of Eh in the stratified paddy field model in 2016 (C -70)

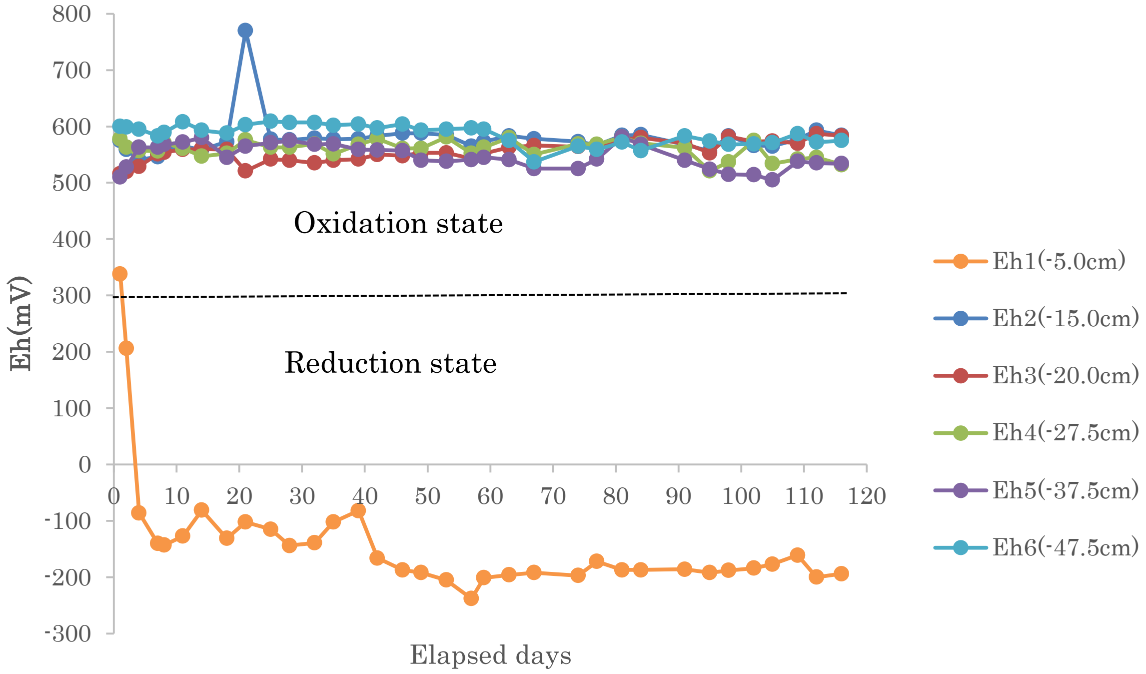


Fig.39 The temporal changes of Eh in the stratified paddy field model in 2016 (O -100)

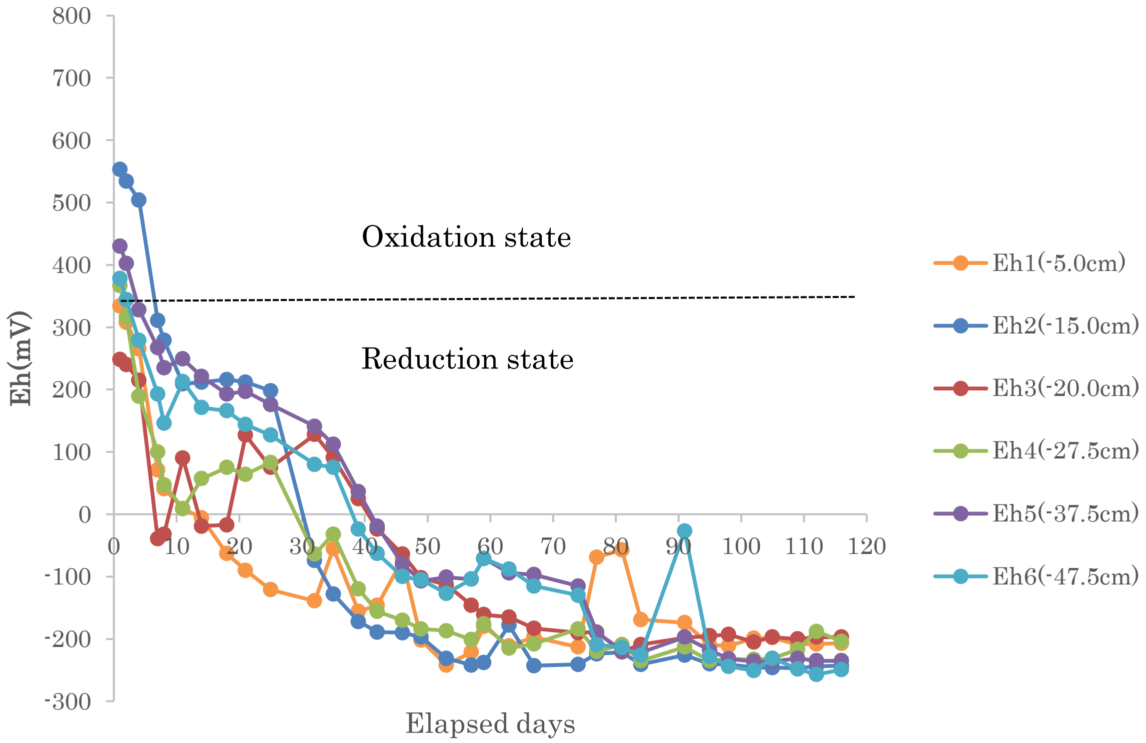


Fig.40 The temporal changes of Eh in the stratified paddy field model in 2016 (C -100)

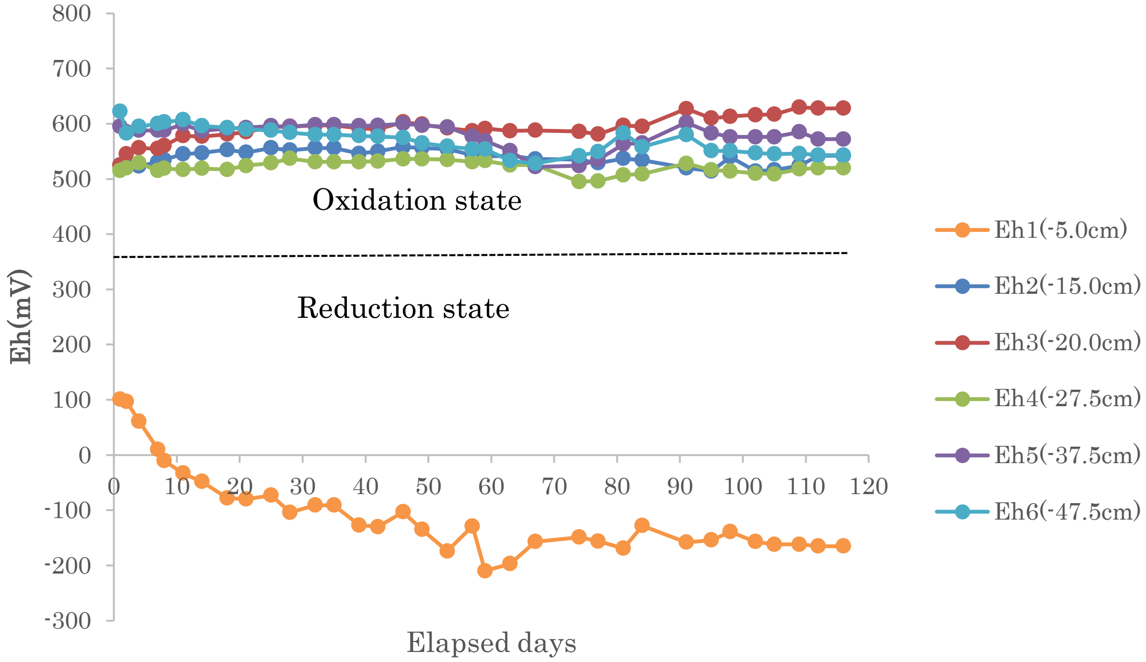


Fig.41 The temporal changes of Eh in the stratified paddy field model in 2016 (O -150)

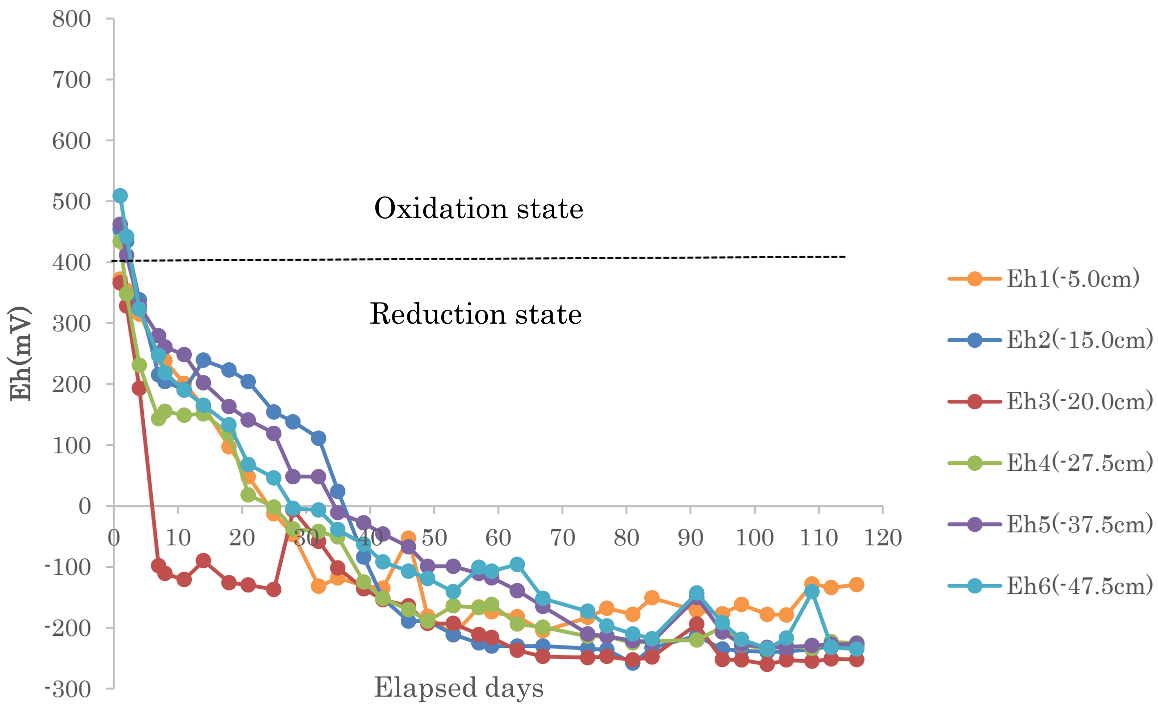


Fig.42 The temporal changes of Eh in the stratified paddy field model in 2016 (C -150)

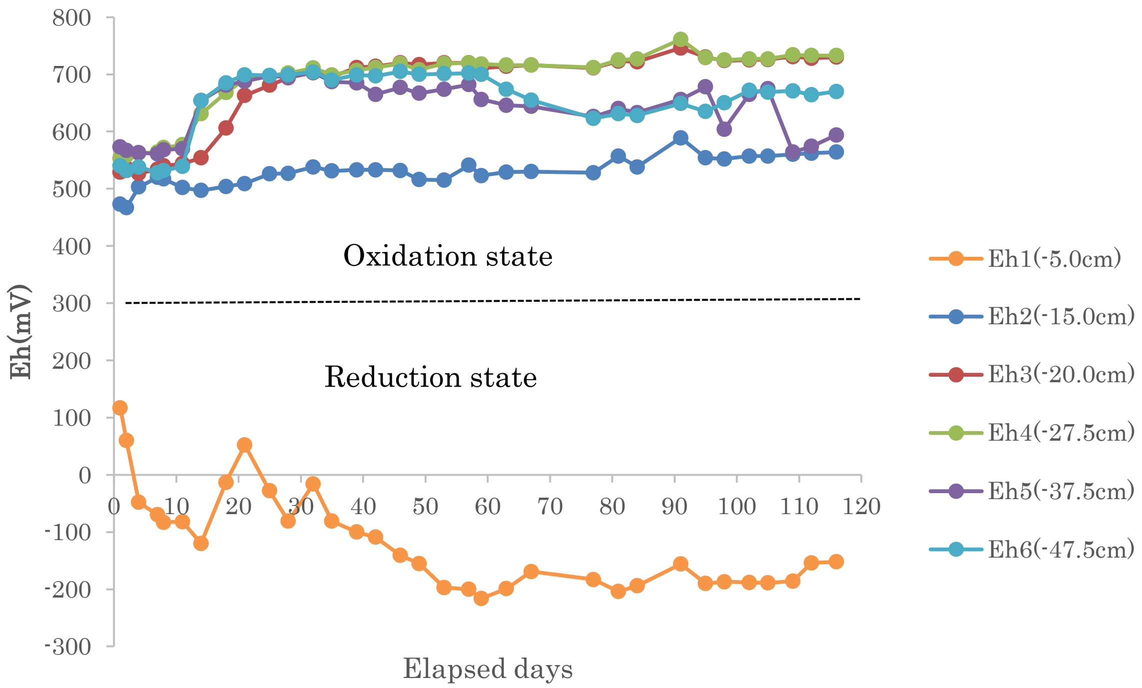


Fig.43 The temporal changes of Eh in the stratified paddy field model in 2016 (O -250)

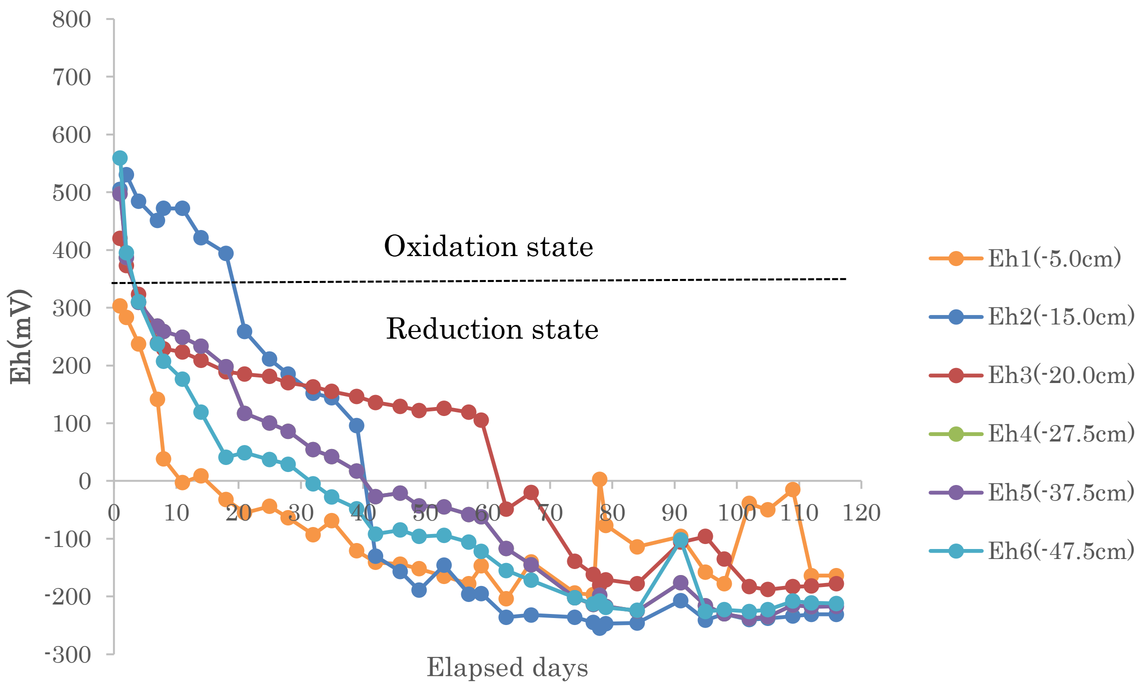


Fig.44 The temporal changes of Eh in the stratified paddy field model in 2016 (C -250)

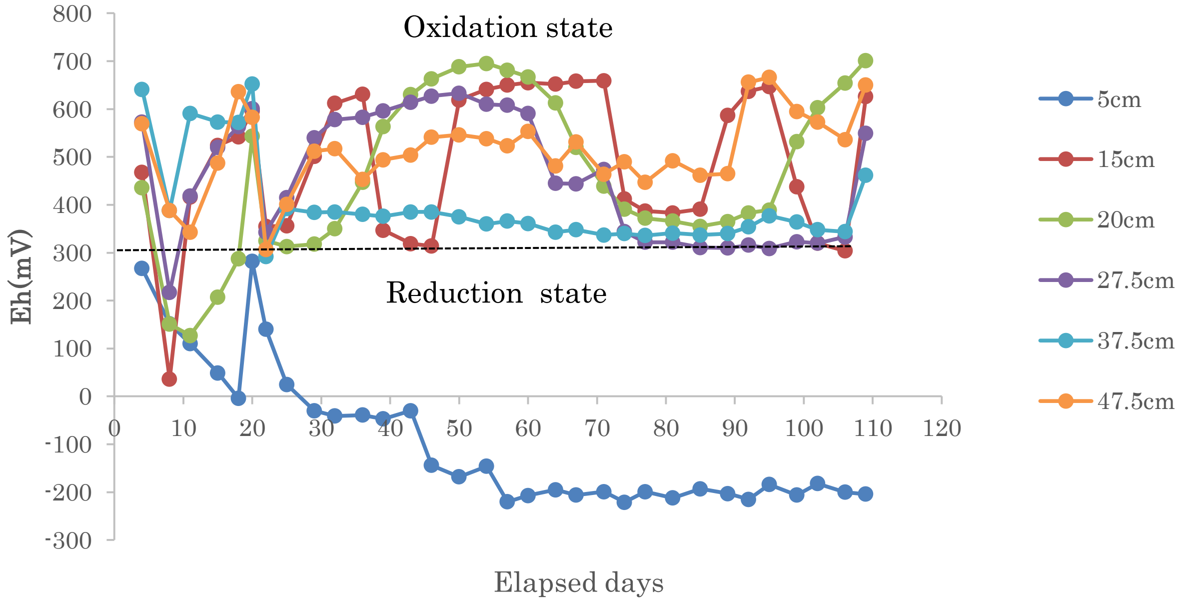


Fig.45 The temporal changes of Eh in the stratified paddy field model in 2017 (O -40)

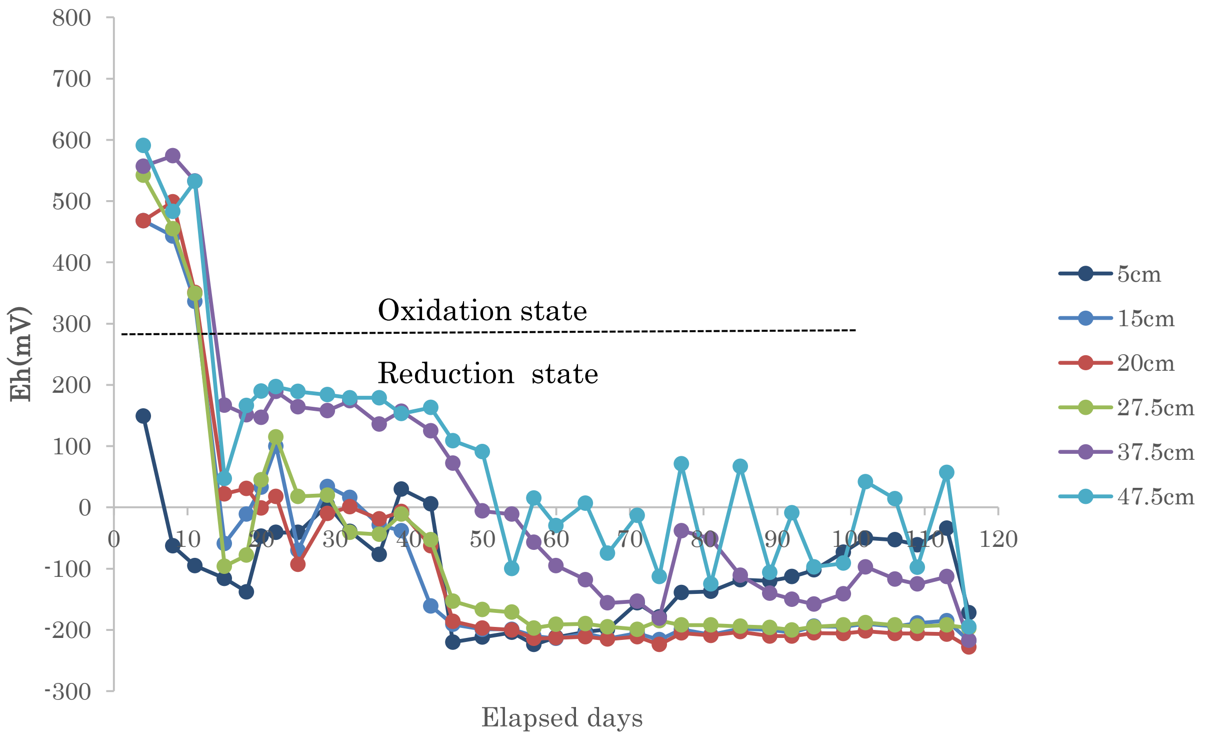


Fig.46 The temporal changes of Eh in the stratified paddy field model in 2017 (C -40)

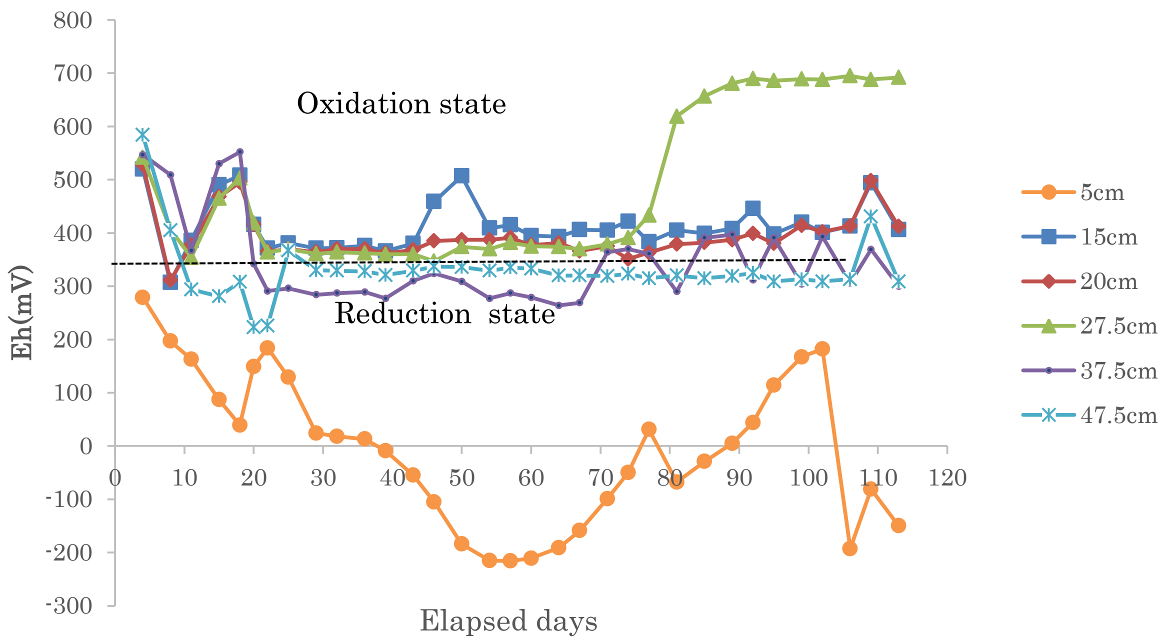


Fig.47 The temporal changes of Eh in the stratified paddy field model in 2017 (O -500)

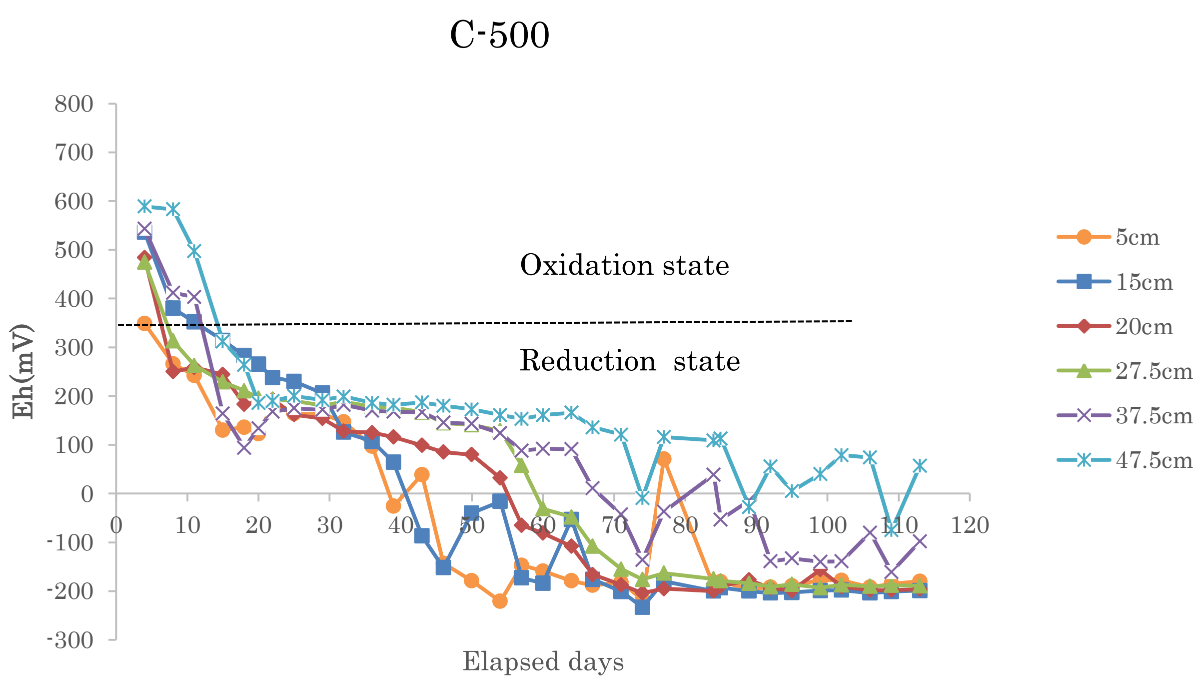


Fig.48 The temporal changes of Eh in the stratified paddy field model in 2017 (C -500)

3.6 Growth

3.6.1 Plant length

The plant length was measured from transplanting to harvesting period. In 2016, the plant length of each model was almost equal, at 90cm~100cm as shown in Fig.46. It has barely increased since 73 days. In 2017, the plant height of each model was almost equal, at 100cm-105cm level as shown in Fig.47. It has barely increased since 70 days. In 2016 and 2017, the average plant length of closed system percolation models was higher than that of open system percolation models.

3.6.2 Number of stem

In 2016, the average number of stem of O-70, C-70, O-100, C-100, O-150, C-150 and O-250, C-250 were 9.4, 9.3, 8.8, 9.3, 8.9, 8.6, 10.0 and 8.3, respectively as shown in Fig 48. In 2017, the average number of stem of O-40, C-40, O-500 and C-500 were 7.8, 8.7, 7.3 and 7.3, respectively as shown in Fig.49. In Both 2016 and 2017 experiment, the number of stem has no significant difference between the open and closed system percolation models.

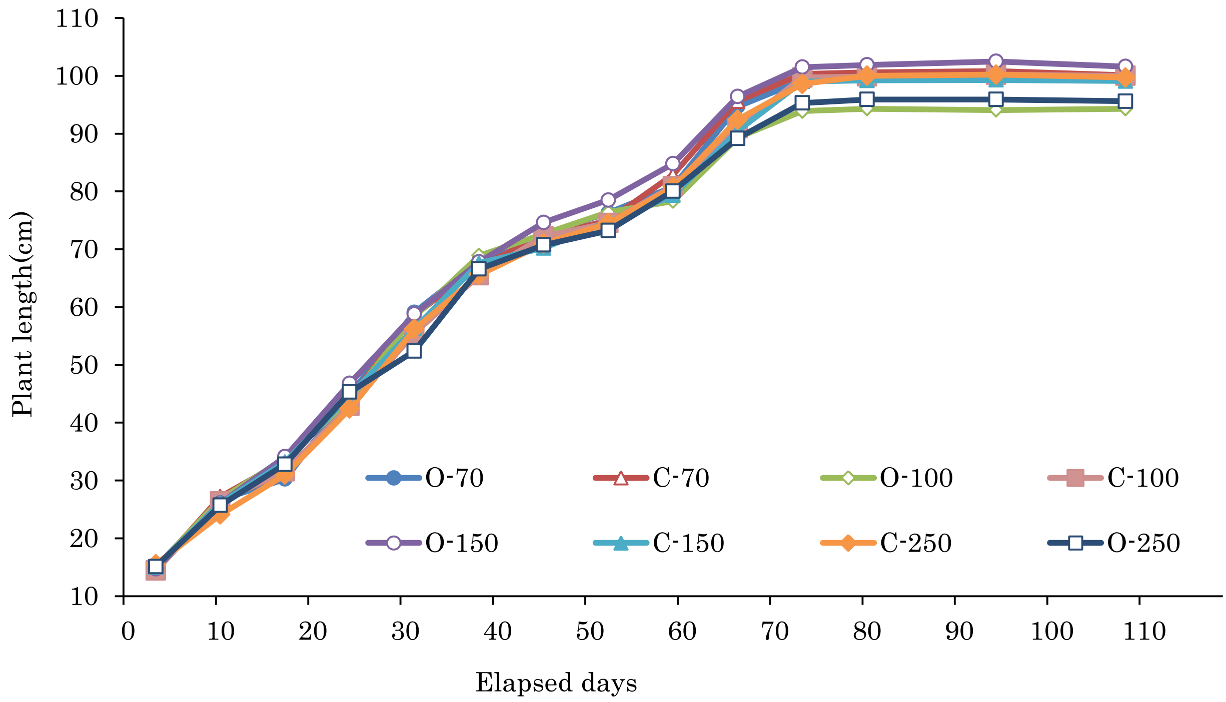


Fig.49 The plant length of rice plant in 2016

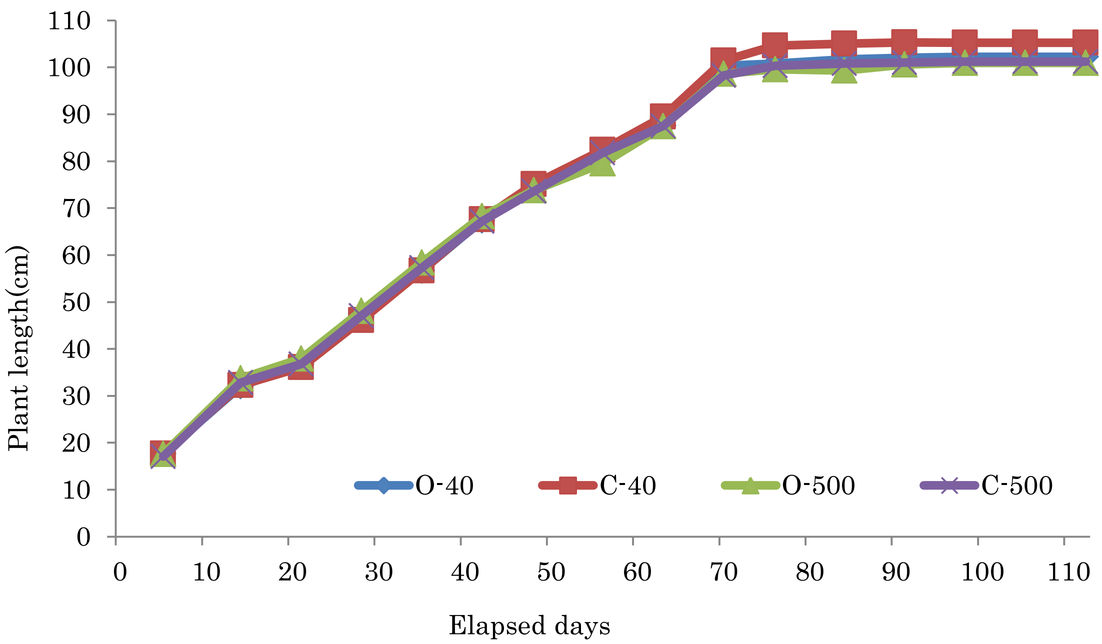


Fig.50 The plant length of rice plant in 2017

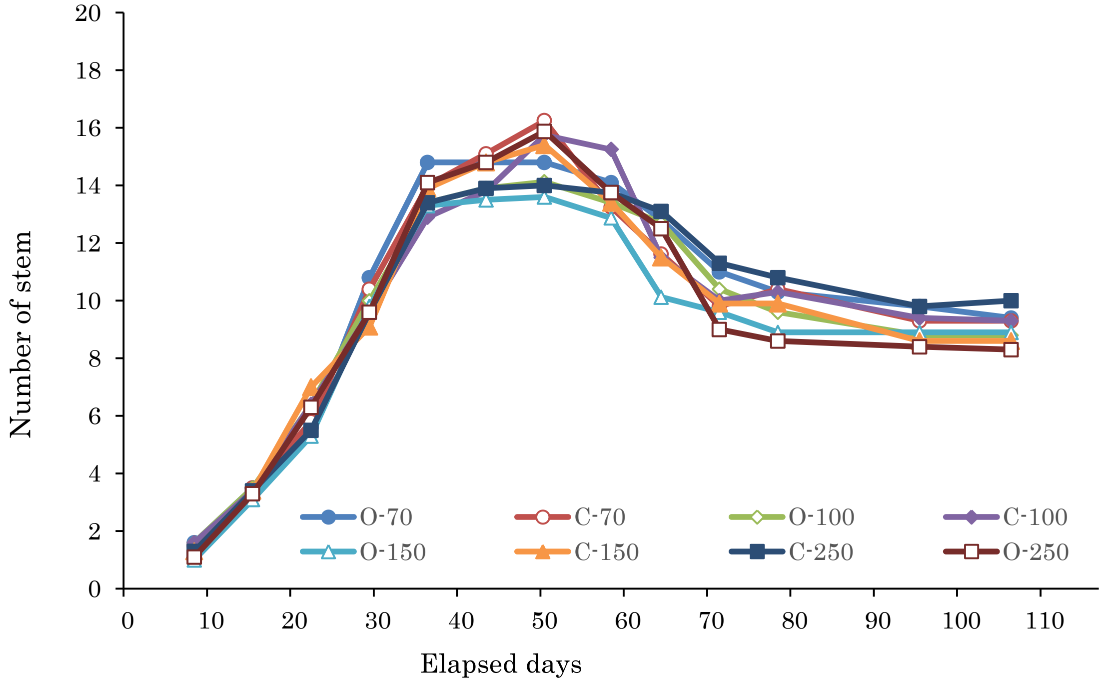


Fig.51 Number of stem in different percolation system in 2016

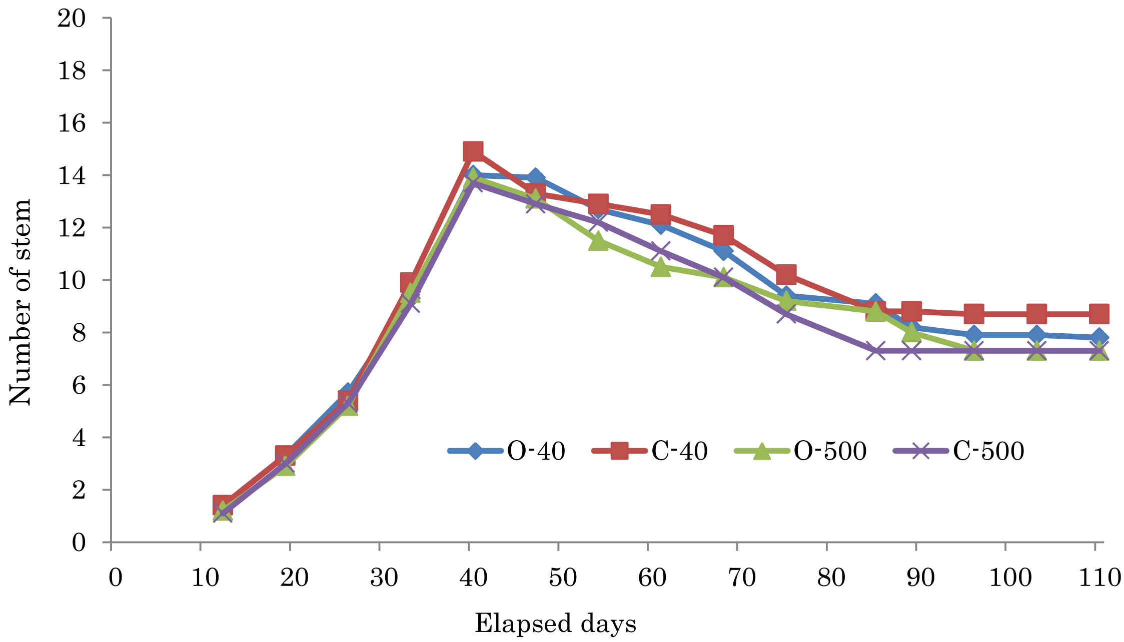


Fig.52 Number of stem in different percolation system in 2017

3.6.3Number of leaves

In 2016, the number of leaf of O-70, C-70, O-100, C-100, O-150, C-150, O-250 and C-250 were 14.3, 14.3, 14.0, 14.4, 14.0, 14.0, 14.3 and 14.0, respectively as shown in Fig.50. In 2017, the number of leaf of O-40, C-40, O-500 and C-500, were all 15.0, as shown in Fig.51. The number of leaves in each system was almost the same in 2016 and 2017.

3.6.4 SPAD (Soil and Plant Analyzer Development)

The chlorophyll meter provides a quick, portable, simple, and non-destructive method for estimating leaf chlorophyll content. In September 15, 2016, the average SPAD value in O-70, C-70, O-100, C-100, O-150, C-150, O-250 and C-250 were 31.6, 34.6, 30.9, 36.2, 31.2, 35.1, 34.8, and 31.9, respectively. In September 14, 2017, the average SPAD value in O-40, C-40, O-500 and C-500 were 33.0, 38.5, 34.8 and 35.8, respectively. In 2016, the SPAD peaked around 24^{th} day, and the highest value was 46.1 in early blooming stage of open and closed system percolation models but that value was gradually decreased with the increasing cultivation period as shown in Fig.52. In 2017, the SPAD peaked around the 35th day, and the highest value was 46.1 in early blooming stage of open and closed system percolation models, but that value also was gradually decreased with the time as shown in Fig.53. In both 2016 and 2017, the average SPAD in closed system percolation was higher than the open system percolation. This may be due to the different effects of Oxidation Reduction state in the soil

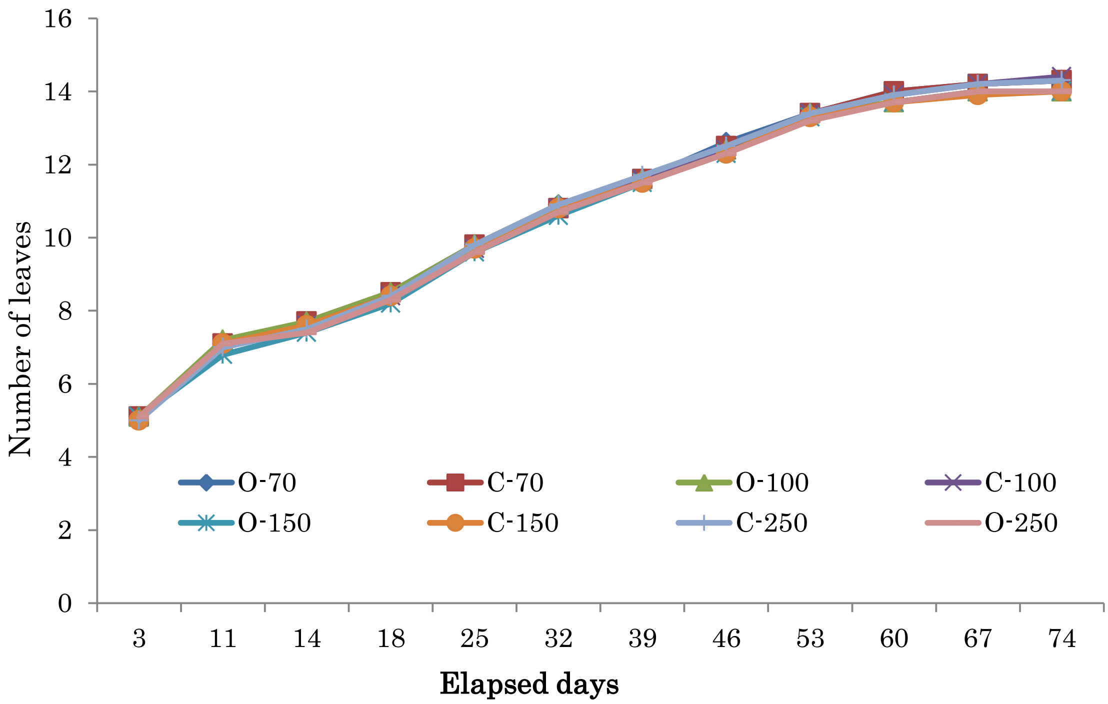


Fig.53 The leaves number of rice plants in different percolation models in 2016

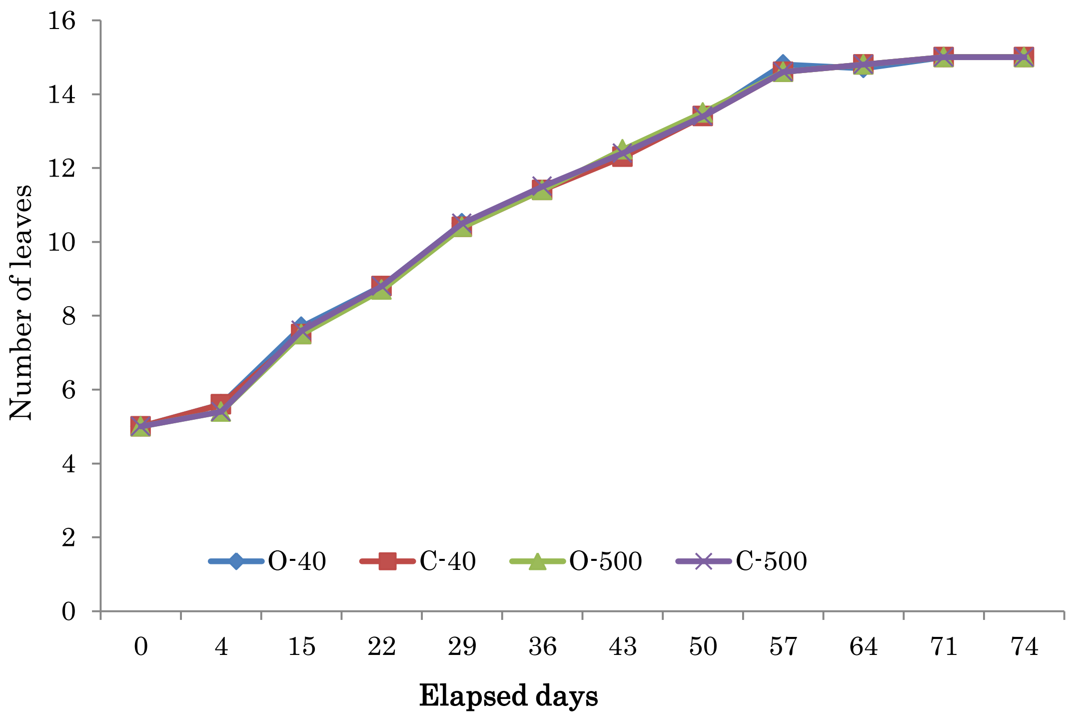


Fig.54 The leaves number of rice plants in different percolation models in 2017

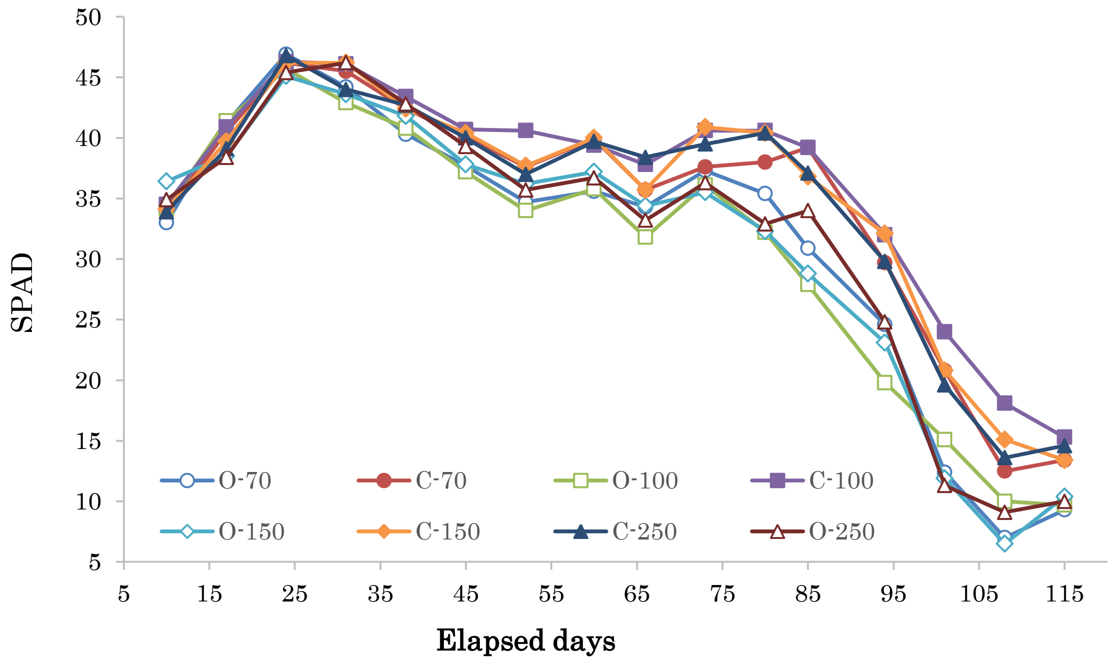


Fig.55 SPAD value of rice plants in the experiment (2016)

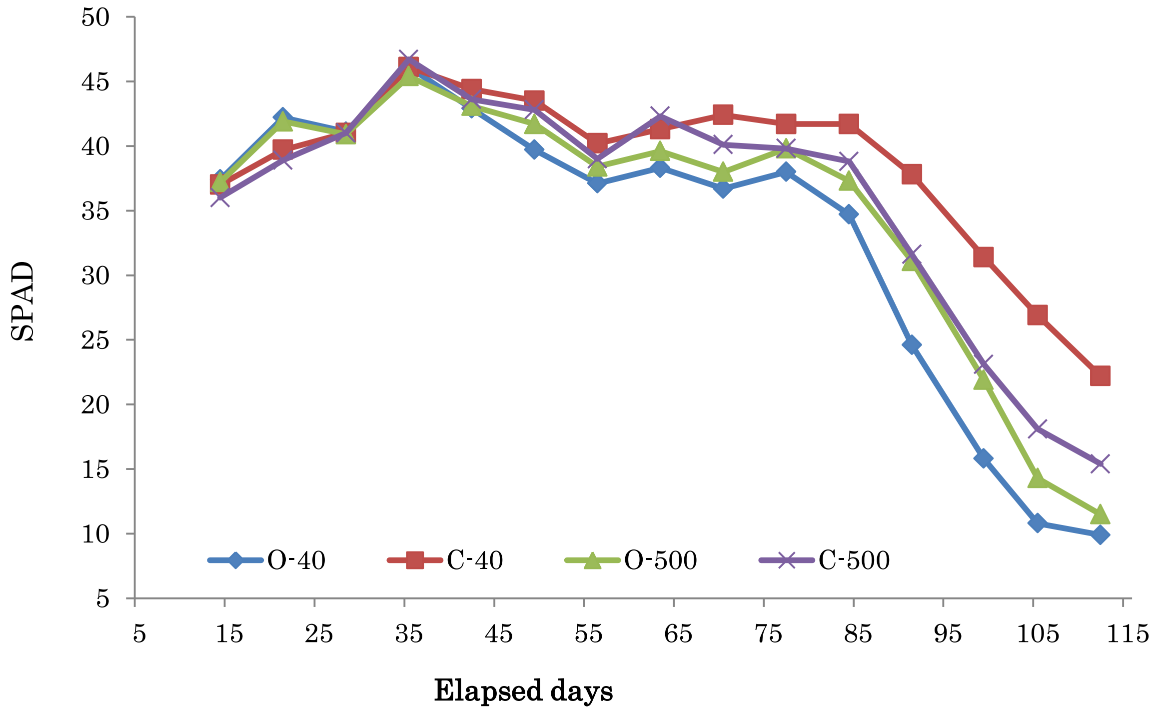


Fig.56 SPAD value of rice plants in the experiment (2017)

3.6.5 The heading period and number of panicle

The heading period and number of panicles were measured of each open and closed system percolation. The heading period of rice plants in both percolations of each model were nearly equal. In 2016 and 2017, heading period of rice plants was started at 66^{th} and 67^{th} from transplanting due to fluctuate of air temperature. The average number of panicle of O-40, C-40, O-70, C-70, O-100, C100, O-150, C-150, O-250, C-250, O-500 and C-500 models were 7.6, 8.3, 9.4, 9.5, 7.6, 9.1, 8.8, 8.6, 8.6, 9.0, 7.0 and 7.1. In this result, the average number of panicle showed in closed-system percolation was higher than the open-system percolation.

3.6.6 Yellowish part of rice plant

The yellowish parts of rice plants were measured in two kinds of percolation systems. In the experiments in 2016 and 2017, the percentage of the Yellow portion of the leaves was first dried in closed system, as shown in Fig.54 and 55. In 2016, at the time of harvesting, the yellowish part of leaf in the closed system was about 59%, while that in the open system was about 33%, and similar results were found in 2017. The results showed that the photosynthetic capacity of rice plants varied greatly in two system at maturity.

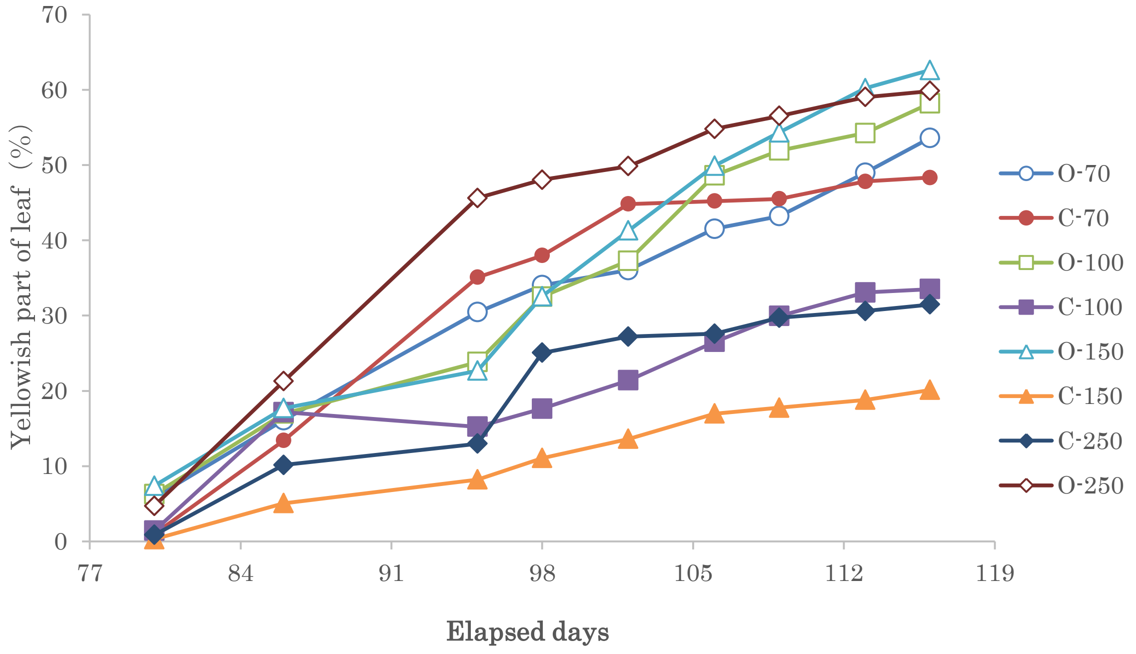


Fig.57 Yellowish part of leaf in open and closed system percolation in 2016

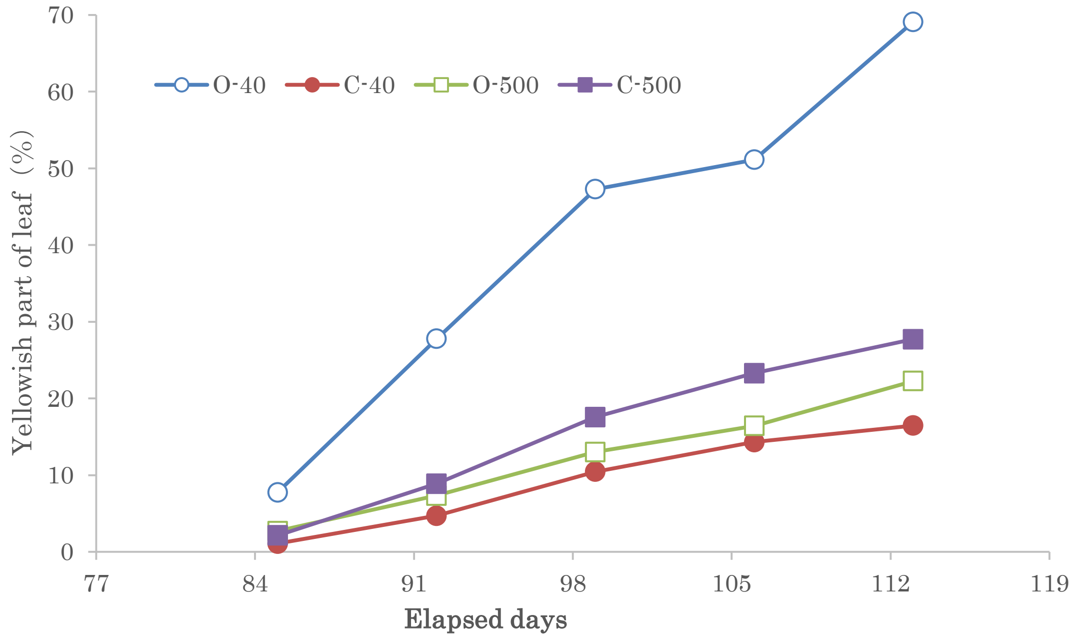


Fig.58 Yellowish part of leaf in open and closed system percolation in 2017

3.6.7 Weight of dried root

The vegetative root distribution of each soil layer were weighted after harvesting in closed and open system percolation models of 2016 and 2017. Rhizosphere is an important environmental interface connecting plant roots and soil. Roots excrete organic acid and nutrients, water and other chemicals, both beneficial and harmful existed in rhizosphere absorbed by roots.. The Weight of dried root in O-70, C-70, O-100, C-100, O-150, C-150, O-250 and C-250 were 19.7, 22.6, 17.0, 21.5, 19.1, 18.7, 18.9 and 20.5(g), respectively, as show the Fig.56. The weight of dried root in O-40, C-40, O-500 and C-500 were 30.0, 27.0, 27.2 and 28.4(g), respectively. as shown in the Fig.57. Weight of dried root of the I and II layer in closed system percolation was higher than the open system percolation. There is only a small amount of little root distributed in the third layer.

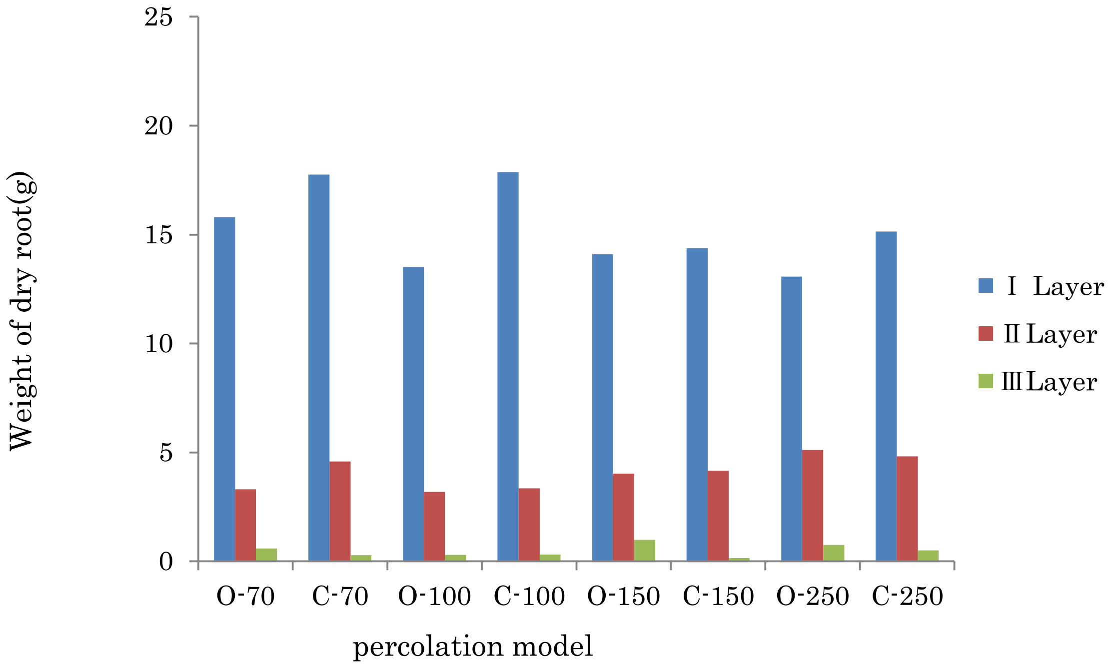


Fig.59 Weight of dry root in different soil layer in 2016

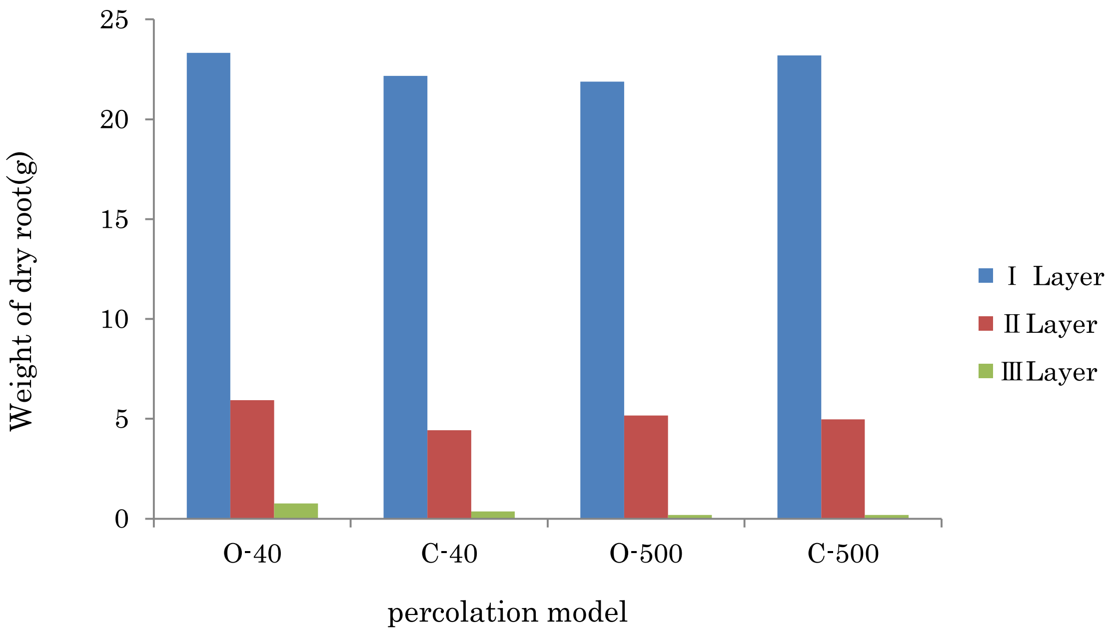


Fig.58 Weight of dry root in different soil layer in 2017

3.7 Yields of rice plants

3.7.1 Dry weight of straw

Vegetative growth of rice plants related to straw weight. The average weight of straw in O-40, C-40, O-70, C-70, O-100, C-100, O-150, C-150, O-250, C-250, O-500 and C-500 were 12, 15.3, 13.3, 15.0, 11.5, 13.9, 13.0, 13.5, 12.3, 13.3, 11.4 and 12.1 g/ hill, respectively, as shown in Figure.58. The above results showed that average straw weight of closed system was higher than that of the open system percolation.

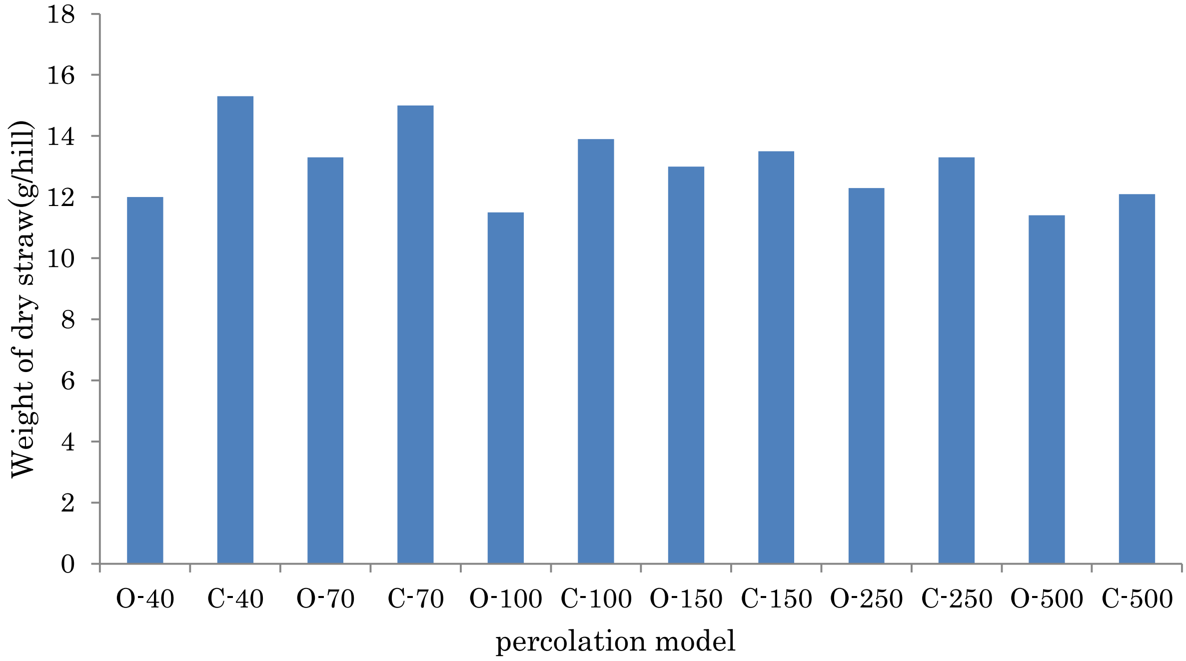


Fig.60 Dry weight of straw in 2016 and 2017

3.7.2 length, Number and Weight of panicle

The average length of panicle of each hill in O-70, C-70, O-100, C-100, O-150, C-150, O-250 and C-250 were 19.2, 19.6, 17.1, 18.7, 19.5, 18.3, 17.6 and 18.7cm in 2016, The average length of panicle of each hill in O-40, C-40 and O-500 and C-500 were 19.3, 18.9, 18.9 and 18.4cm in 2017, respectively as shown in Table 2. The average number of panicle of closed system percolation were higher than the open system percolation. Moreover, Weight of panicles in open system percolation were lower than the closed system percolation.

Table 2 length, number and weight of Panicles in experimental models.

Year	Model	Length of panicle (cm)	No. of Panicles (Panicles/hill)	Weight of panicles (g)
2016(n=8)	O-70	19.2±1.8[a]	9.4±1.1[a]	1.9±0.3[a]
	C-70	19.6±1.1[a]	9.6±1.2[a]	2.0±0.2[a]
	O-100	17.1±1.7[b]	8.5±1.8[a]	2.2±0.6[a]
	C-100	18.7±1.0[ab]	9.5±2.5[a]	2.2±0.3[a]
	O-150	19.5±1.5[a]	8.8±0.7[a]	2.0±0.2[a]
	C-150	18.3±1.1[ab]	8.9±2.4[a]	2.2±0.3[a]
	O-250	17.6±1.0[ab]	8.6±1.3[a]	2.0±0.2[a]
	C-250	18.7±1.0[ab]	9.6±1.8[a]	2.2±0.3[a]
2017(n=10)	O-40	19.3±1.6[A]	7.8±1.8[A]	2.6±0.3[A]
	C-40	18.9±1.2[A]	8.5±1.4[A]	2.6±0.2[A]
	O-500	18.9±1.3[A]	7.3±1.6[A]	2.4±0.3[A]
	C-500	18.4±1.0[A]	7.0±1.6[A]	2.5±0.2[A]

Note: Tukey-Kramer test was performed at 5% level; letter indicates significant difference. The numerical value of ± shows standard deviation

3.7.3 The number of panicles, the weight of one panicle and the number of rice grains

The average number of panicles per unit hill in each model was 8.5～9.6 /hill in 2016. The average number of panicles per unit hill in each model was 7.0～8.5 /hill in 2017. Likewise, the averages of the weight of one panicle and the number of grains of brown rice per unit hill were 1.9～2.2 g /panicle(2016), 2.4～2.6 g /panicle(2017) and 553～710 grains /hill(2016), 600～734 grains/hill(2017), respectively. In addition, the averages of the percentage of ripening and the 1,000 grain weight of brown rice were 86.5～94.3%(2016), 97.0.～97.5%(2017) and 18.9～19.9g(2016), 22.7～23.3g(2017), respectively. No significant differences were found in any of the items of the models with different percolation types. Paul et al. (2011a) reported that yield components of the closed-system percolation model were significantly higher than those of the open-system percolation model though their experiment was conducted by using a different soil type for Cd polluted soil. Sasaki et al. (2016a) conducted an experiment using stratified paddy field models with a Cd polluted soil layer and reported that there was no significant difference between the models with different percolation patterns. Soil dressing was presumed to be one of the reasons why no significant difference was observed in that study. In a previous study (Shibuya, 1979), it was reported that Cu concentrations affected the number of panicles and the percentage of ripening. In this study, however, the influence of the Cu concentrations on the growth of rice plants was not remarkable, which may be attributed to the soil dressing.

Table 3 The weight of one panicle, No.of Panicles, The percentage of ripening, The number of brown rice per unit hill, The 1000 grain weight of brown rice (mg/kg)

Year	Model	The weight of one panicle (g)	No.of Panicles (panicles/hill)	The percentage of ripening (%)	The number of brown rice per unit hill (grains/hill)	The 1000 grain weight of brown rice (g)
2016(n=8)	O-70	$1.9±0.3^{a}$	$9.4±1.1^{a}$	$92.3±2.2^{a}$	$644.3±116.0^{a}$	$19.1±0.9^{a}$
	C-70	$2.0±0.2^{a}$	$9.6±1.2^{a}$	$86.5±4.1^{b}$	$616.9±118.8^{a}$	$19.7±0.5^{a}$
	O-100	$2.2±0.6^{a}$	$8.5±1.8^{a}$	$94.0±2.2^{a}$	$553.2±133.2^{a}$	$19.2±0.6^{a}$
	C-100	$2.2±0.3^{a}$	$9.5±2.5^{a}$	$93.6±2.8^{a}$	$710.4±170.4^{a}$	$19.3±0.6^{a}$
	O-150	$2.0±0.2^{a}$	$8.8±0.7^{a}$	$93.8±1.4^{a}$	$642.9±103.9^{a}$	$18.9±0.3^{a}$
	C-150	$2.2±0.3^{a}$	$8.9±2.4^{a}$	$94.3±2.3^{a}$	$670.4±128.1^{a}$	$19.6±0.3^{a}$
	O-250	$2.0±0.2^{a}$	$8.6±1.3^{a}$	$93.7±1.5^{a}$	$627.9±65.0^{a}$	$19.4±0.8^{a}$
	C-250	$2.2±0.3^{a}$	$9.6±1.8^{a}$	$93.1±1.5^{a}$	$680.5±163.9^{a}$	$19.9±0.8^{a}$
2017(n=10)	O-40	$2.6±0.3^{AC}$	$7.8±1.8^{A}$	$97.0±0.9^{A}$	$669.1±88.7^{A}$	$23.1±0.4^{A}$
	C-40	$2.6±0.2^{A}$	$8.5±1.4^{A}$	$97.3±0.9^{A}$	$734.0±137.4^{A}$	$23.3±0.5^{A}$
	O-500	$2.4±0.3^{AB}$	$7.3±1.6^{A}$	$97.5±0.6^{A}$	$600.9±128.5^{AB}$	$22.7±0.6^{AB}$
	C-500	$2.5±0.2^{AC}$	$7.0±1.6^{A}$	$97.5±0.7^{A}$	$601.7±138.2^{AB}$	$23.2±0.7^{A}$

Note: Tukey-Kramer test was performed at 5% level; letter indicates significant difference. The numerical value of ± shows standard deviation

3.8 Concentration of copper in roots

The results of Cu concentrations in rice plants are listed in Table 4.The values of the open system were larger than those of the closed system. Regarding Cu concentration in stems and leaves except for O-100 and C-100, the values of the open system were greater than those of the closed system. No significant differences were found in stems and leaves of models between different percolation types.

The relationship of copper concentration between in soil and in rice grains of open and closed -system are shown in Fig.59. Cu concentration in rice grains of open-system were higher than those of closed-system regardless of soil Cu concentration. Copper concentration in rice grains did not increase significantly with the increase of soil Cu concentration.

The relationship of copper concentration between in soil and in stem and leaves is shown in Fig.60. As can be seen from the figure, the values of open system are always above those of the closed system. Even if Cu concentration in soil increased, that in stems and leaves did not change significantly.

The graph of copper concentration in soil and in root are shown in Fig.61. As can be seen from the figure, copper concentration in root increased with the increase of copper concentration in soil.

Table 4 Copper concentration in Rice grains, stems and leaves, root

(mg/kg)

Year	Model	Rice grains	stem and leaves	Root of plow layer
		n=10	n=5	n＝5
2016	O-70	4.3±0.3^{ad}	1.5±0.4^{abc}	17.1±2.1^{ac}
	C-70	3.4±04^{b}	1.24±0.4^{abc}	19.6±1.1^{ab}
	O-100	4.0±0.4^{a}	1.2±0.4^{abc}	16.4±1.3^{c}
	C-100	3.3±0.2^{bc}	1.2±0.1^{abc}	14.9±0.7^{cd}
	O-150	4.6±0.7^{d}	1.8±0.4^{a}	19.8±1.6^{b}
	C-150	3.3±0.3^{b}	1.1±0.4^{cd}	20.2±0.9^{b}
	O-250	4.1±0.4^{ad}	1.6±0.2^{ac}	13.7±0.5^{d}
	C-250	2.8±0.2^{c}	0.8±0.3^{bd}	20.3±1.4^{b}
		n=7	n=5	n＝5
2017	O-40	3.6±0.3^{A}	2.4±0.3^{A}	14.3±0.6^{A}
	C-40	2.5±0.2^{B}	2.3±1.0^{AB}	13.0±2.3^{A}
	O-500	3.7±0.2^{C}	2.1±0.1^{AB}	15.3±1.2^{A}
	C-500	2.8±0.1^{AB}	1.3±0.2^{B}	16.3±0.9^{A}

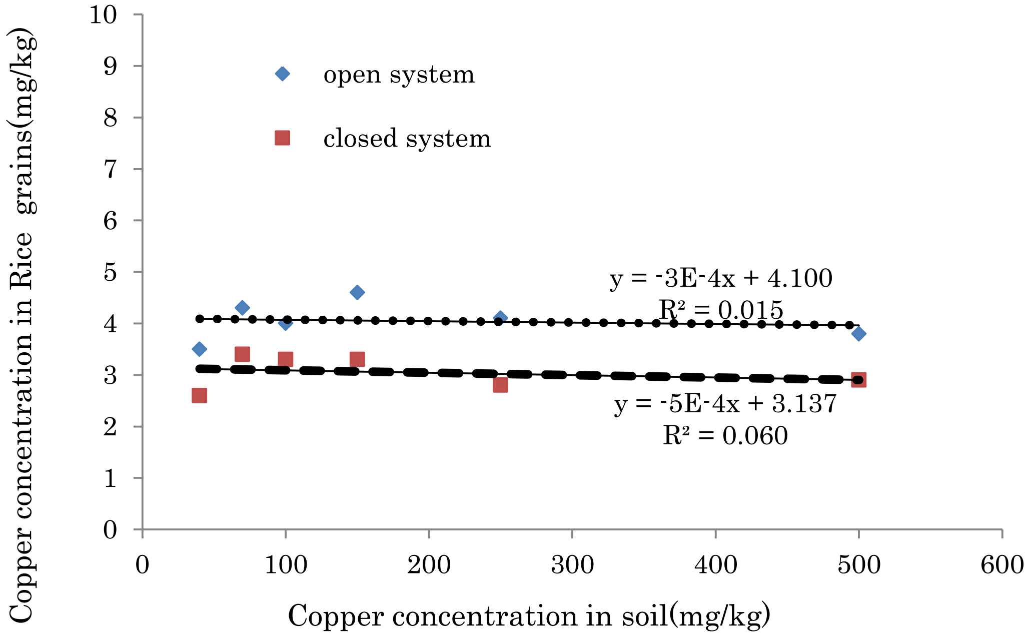


Fig 61 Comparison between copper concentration in soil and in Rice

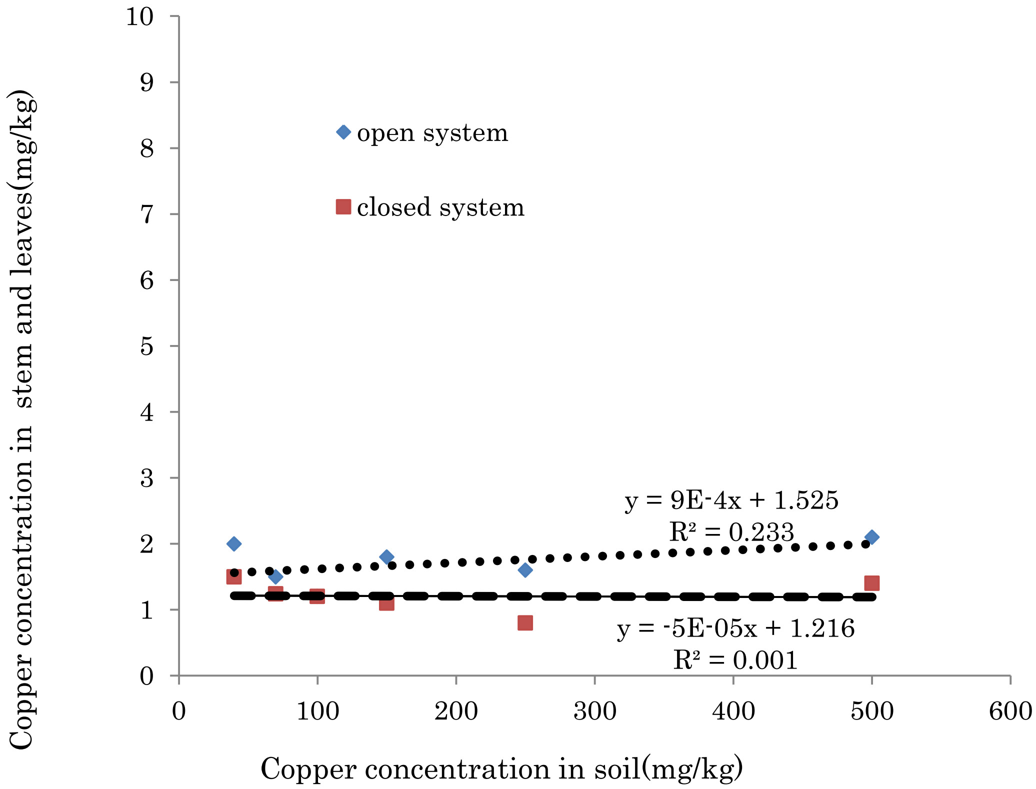


Fig 62 Comparison between copper concentration in soil and in stem and leaves

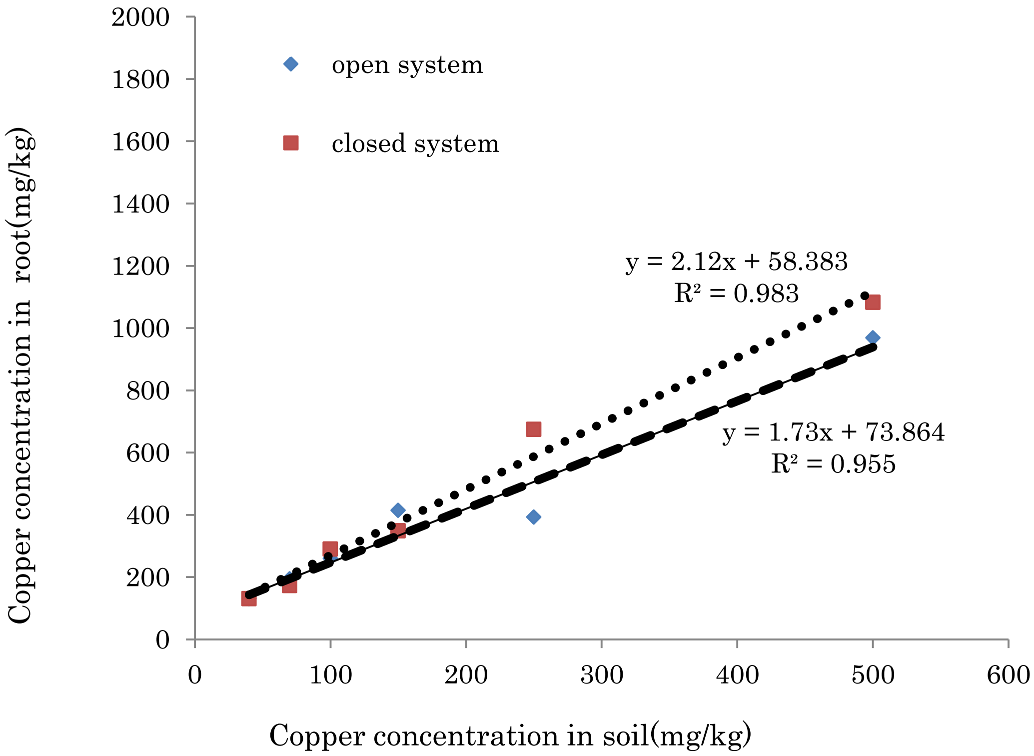


Fig 63 Comparison between copper concentration in soil and in root

Chapter 4

Summary and conclusion

In this study, we investigated the effects of percolation patters and Cu concentration of polluted soil on growth and yield of rice plants and Cu uptake by using stratified paddy field models. From the experiments, we reached following conclusions (Fan et al, in press)

Copper Concentrations in Rice Plants

Rice Grains: Cu concentrations in brown rice ranged from 2.8 to 4.3 mg/kg (2016) and 2.6 to 3.8 mg/kg（2017）which were similar to the values reported by Asami (2010). Their studies, however, did not make a distinction between open and close percolation patterns. Paul et al. (2011b) reported that the significant difference was observed in Cu concentrations in brown rice due to differences in percolation patterns. In addition, in their study, despite the low soil Cu concentrations, Cu concentrations in brown rice ranged from 2.5 to 4.2 mg/kg, similar to the results of our experiment. Thus the Cu concentrations in the lower layer did not make much difference in Cu concentrations in brown rice. From these results, it was revealed that the differences in Cu concentrations in brown rice due to differences in percolation patterns were confirmed as those in Cd concentrations found in the experiments conducted by Sasaki et al. (2016a, b). In our study, Cu concentration in rice grains of open system were higher than those of close system regardless of soil Cu concentration. Copper concentration in rice grains did not increase significantly with the rise of soil Cu concentration.

Stems and Leaves: It confirmed that statistically significant differences in the Cu concentrations in stems and leaves were between O-150 (1.8 mg/kg) and C-150 (1.1 mg/kg), O-250 (1.6 mg/kg) and C-250 (0.8 mg/kg). However, there was no significant difference between O-40(2.4 mg/kg) and C-40(2.3 mg/kg), O-70(1.5 mg/kg) and C-70(1.2 mg/kg), O-100(1.2 mg/kg) and C-100(1.2 mg/kg), O-500(2.1 mg/kg) and C-500(1.3 mg/kg). This discrepancy might be caused by the soil Cu concentrations, but this requires further elucidation. the open system is always above the closed system. As Cu concentration in soil increased that in stems and leaves did not change significantly.

Roots: Cu concentrations in roots were 2-3 times the larger than those in soil in Table 4. This may be due to the absorption of Cu in soil for the whole growing period by roots. Here, further discussion is needed to clarify its mechanism as in the case of stems and leaves.

Cu concentrations in the rice plants were in the order of roots > brown rice > stems and leaves. Their ratio was about 1:3:15. This order was similar to those of Shibuya (1979) and Paul et al. (2011b) who used rice plants, and to those of Li et al. (2017) who used soybeans. copper concentration in root increased with the increase of copper concentration in soil.

Unlike the rice grains, and stems and leaves, Cu concentration in roots significantly increased with the increase of soil Cu concentration.

Growth and yield of rice plants

Growth of rice plants: The average plant length of each model was almost equal, at 90 ~100cm level(2016) and 100cm level(2017). No. of Panicles each model was about 9 Panicles /hill (2016) and 8 Panicles /hill (2017), showing a small difference between them. Weight of panicles was 2 Panicles /hill(2016) and 2.5 Panicles /hill(2017). No significant difference was observed in the plant length, No. of Panicles and Weight of panicles regardless of the percolation patterns. Previous research (Shibuya, 1979) reported that Cu concentrations in the Cu polluted soil layer had an influence on the growth of rice plants. In this study, however, influence of the Cu concentrations on the growth of rice plants was not noticeable, which may well to have resulted from the application of soil dressing.

Yield of rice plants: An average number of panicles per unit hill in each model was 8.5～9.6 /hill(2016) and 7.0~8.5/hill (2017). Likewise, averages of the weight of one panicle and the number of grains of brown rice per unit hill were 1.9～2.2 g / panicle ,616～680 grains /hill(2016) and 2.4~2.6 g / panicle, 600~734 grains /hill(2017), respectively. In addition, averages of the percentage of ripening and the 1,000 grain weight of brown rice were 86.5～93.7% ,19.1～19.9g(2016) and 97.0~97.5%,22.7~23.3g(2017), respectively. No significant differences were found in any of the items of the models with different percolation types. Paul et al. (2011a) reported that yield components of the closed-system percolation model were significantly higher than those of the open-system percolation model though their experiment was conducted by using a different soil type for Cd polluted soil layers. Sasaki et al. (2016a) conducted an experiment using stratified paddy field models with a Cd polluted soil layer and reported that there was no significant difference between the models with different percolation patterns. Soil dressing was presumed to be one of the reasons why no significant difference was observed in that study. In a previous study (Shibuya, 1979), it was reported that Cu concentrations had an influence on the number of panicles and the percentage of ripening. In this study, however, the influence of the Cu concentrations on the growth of rice plants was not remarkable, which may be attributed to the soil dressing.

Using twelve types of Cu polluted stratified paddy field models, we conducted an experiment to clarify the effects of percolation patterns in the sub-layer (both plowsole and subsoil) on the Cu concentrations in the rice plants and their growth and yield. For the Cu polluted soil layer, two different Cu concentrations of 40，70，100，150，250 and 500 mg/kg were prepared.

The results of our experiment showed that in the open system percolation models the sub-layers became oxidation layers and those in the closed system percolation models the sub-layers became reduction layers. Cu concentrations in the brown rice of the open system percolation models were significantly larger by 5% than those of the closed system percolation models. In the models with concentrations of Cu (150mg/kg and 250mg/kg), the Cu concentrations in stems and leaves showed significantly different values between the percolation patterns. However, there was no statistically significant difference in the growth and yield of rice plants between the percolation patterns.

Under the above conditions, difference in percolation patterns of the stratified paddy field models did not affect the growth and yield of rice plants, while it had an influence on the Cu concentration in the rice plants.

Chapter5

Future Research Plan

From the above, here are further problems that should be discussed in future research.

① Contaminated apple orchards

More than 1000 mg/kg of high concentrations of copper contaminated soil experiment study is necessary

② Compound contaminated soil

About the influence of different infiltration patterns of Cd and Cu on the growth and harvest of rice and the absorption of Cu and Cd

③ Other heavy metal pollution

The percolation pattern can be evaluated with arsenic, Zinc, Nickel and Cobalt polluted soil.

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